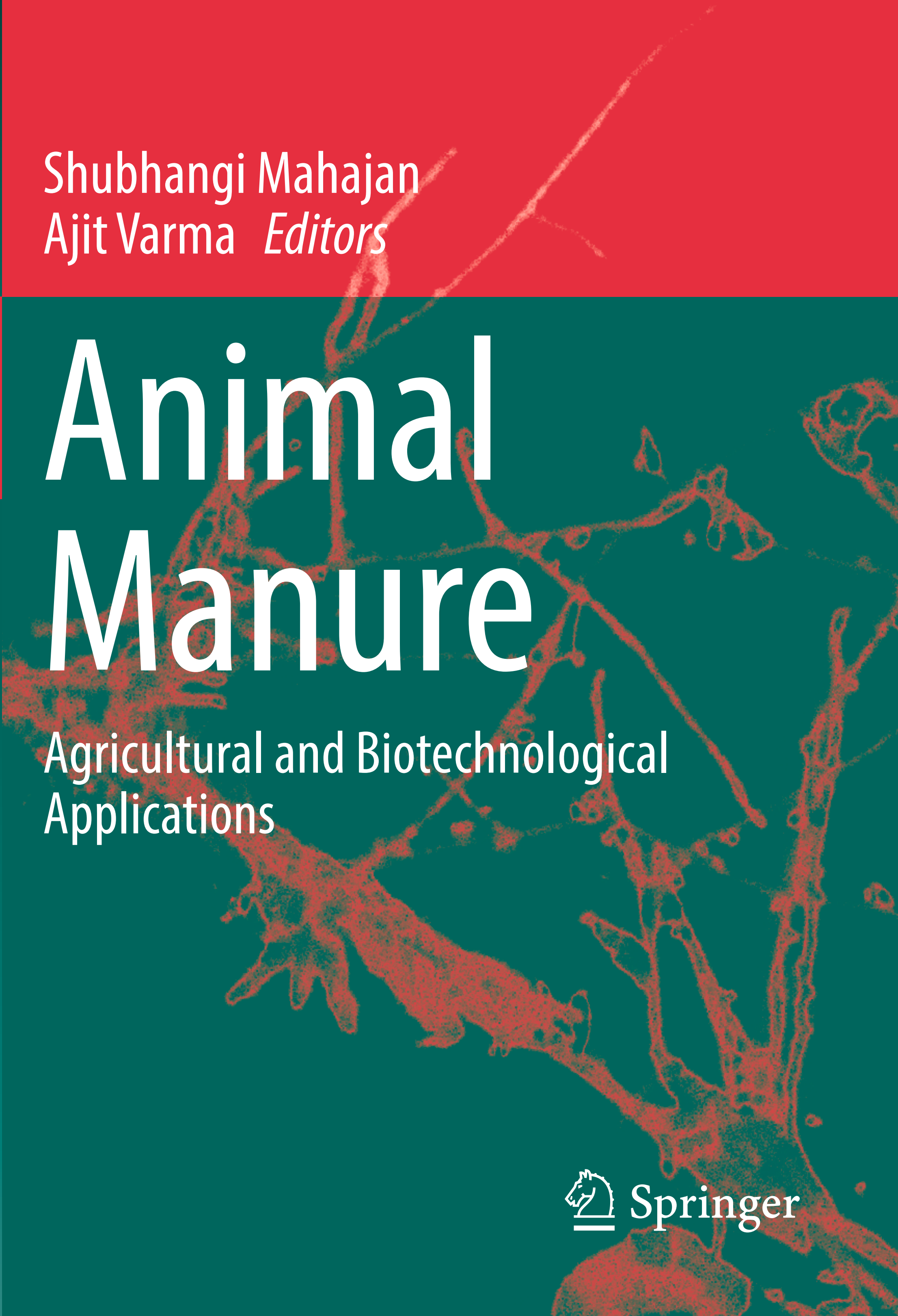
Soil Biology

Shubhangi Mahajan
Ajit Varma *Editors*

Animal Manure

Agricultural and Biotechnological Applications



Springer

Soil Biology

Volume 64

Series Editor

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Editors

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ISSN 1613-3382 ISSN 2196-4831 (electronic)
Soil Biology
ISBN 978-3-030-97290-5 ISBN 978-3-030-97291-2 (eBook)
https://doi.org/10.1007/978-3-030-97291-2

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This book is dedicated to Ms. Rani Mahajan
who has given birth to a talented, intelligent,
and most beautiful Ms. Shubhangi Mahajan

Foreword

For better health and nutrition, organic food is preferred globally by health-conscious people. Accordingly, the area under organic farming is increasing all over the world. For organic farming, availability of quality animal manure becomes important. In this context, the manure nutrients such as phosphorus (P), nitrogen (N), and metals sometimes exceed the needs of plant growth and hence result in environmental pollution. On the contrary, presence of macro- and micro-elements enhances the overall soil fertility. Hence, while increasing agricultural production, the manure nutrients need to be managed efficiently through biotechnological options to improve soil health and fertility without any adverse impact on environment. Biotechnology has helped industry to develop plant-, animal-, microbe-, and diet-based approaches to alleviate potential adverse effects of excessive manure nutrients on water, air, and soil quality, thus helps both agriculture and better human living. Management of products from animal manure and their effectiveness, including societal acceptance, is highly dependent on the biotechnological implications and the enabling policy environment by the government. Also, it is a fact that new biotechnology-related techniques will continue to be evolved and applied to manage better the animal manure nutrients.

We are aware that nature of manure nutrients and soil conditions affect diversity of microbes and their interaction with crop for improved production, an approach that is important for agricultural sustainability. The manure amended quorum of microbes is directly or indirectly involved in soil fertility and plant growth promotion. The deployment of animal manure is thus a sustainable approach for maintaining crop yields under organic farming. The book entitled **"Animal Manure: Agricultural and Biotechnological Applications"** has 19 chapters which deal with biotechnology of rumen microorganisms, process of methanogenesis, mitigation strategies, rumen microbiology and microbial degradation, structure and function of rumen microbes and exploring their biotechnological applications, potential of cow dung in sustainable agriculture and environmental health, role of novel microorganisms in producing biogas, bioenergy from animal manure and animal manure for soil fertility, bioremediation, cow dung as domestic fuel and better soil health management, cow manure for clean energy, role of beneficial microorganisms, strategies for enteric methane mitigation, assessment of carbon emission, and biotechnological applications in animal nutrition. This book thus has a wealth of information to unfurl animal manure in the form of a compendium.

I congratulate Professor Ajit Varma and his colleagues for their insightful publication on animal manure in relation to agro-biotechnological implications to promote organic farming while ensuring ecosystem resilience. I am sure this book with wealth of knowledge covering diverse aspects will be highly useful to the academicians, research scholars, policymakers, and those interested in improving soil health and increased agricultural production in a more sustainable manner.

Indian Council of Agricultural Research, R. S. Paroda
New Delhi, India

Preface

DBT-BIRAC Award for Innovative Research Project. Honoured by the Minister of Science and Technology, Govt of India, Dr. Harsh Vardhan to Ms. Shubhangi Mahajan.

Eminent scientists further suggested to focus the research attention on animal health, their physiology and their biotechnological applications. It is believed that important, innovative, and novel ideas emerge over a cup of Tea or glass of Beer and the weather must be congenial and most suitable for materialization of original ideas. The genesis of this book underlines the concept developed in the late 2020.

Compost (dung manure) is widely used in Indian agriculture, indicating its potential for increasing crop output. Considering many uses of cow dung, the scientific community is becoming increasingly interested in its scientific analysis and use, especially now that organic farming and indigenous medicines are garnering a lot of attention across the world.

The Vedic civilization of India extends back several thousand years, and it has spread throughout the world as a result of the Indus Valley's influence on people's lives and cultures. The learned Rishis, Seers, and Sages of Vedic India have left behind a treasure trove of knowledge in the form of the Vedic texts, which were compiled as the four Vedas by Maharishi Vedavyasa during the Mahabharata period some 5000 years ago and are still the most ancient texts composed in a natural scientific language, Sanskrit, which is also the mother tongue of many Asian and European languages. The Vedas acknowledge "Cow" as a sacred "holy mother", a saviour of life and an embodiment of all gods and goddesses that bestow the bliss of havens on earth. The cow breed Kamdhenu has been referred by the Vedic Seers as the most sacred Cow as per the Gou-mantra.

In India, cow dung is utilized as an environmental preservative, disinfectant, source of humic acid elements to improve organic fertilizer quality, and pest repellent in rural households. Cows provide people with milk, yoghurt, cheese, butter, and "ghee", among other things. Cow's milk is like ambrosial nectar, according to Vedanta, and ghee prepared from cow's milk is the best of all. Advances in cow science have made it easier to analyse scientific evidence of potential applications of directly and indirectly generated cow products that contribute to long-term environmental health. Cow worship in India has a long history dating back to the Vedic era, several thousand years ago. Cow being a domestic animal, whose milk is as nourishing as one's mother's milk, and whose utility in a primarily agricultural civilization like India made it a highly beneficial animal whose dung and urine played a key role in Indian medicine system as well as ancient India's religious rituals. *Ayurveda* and *Charaka Samhita* are the examples of Indian writings having detailed description of immense medical, health, and general well-being benefits of various products obtained from cow. Cow products such as milk, yoghurt, refined butter ghee, and even cow dung were regarded as highly useful medicines in ancient India's medicine system and till date continue to be utilized in Indian household as medicines of great value. Cow products are examined in modern science as well, and have been discovered to be quite effective in the treatment and prevention of numerous ailments.

It has been revealed that ruminants retain the competence to produce the greatest level of methane through the action of methanogens microorganisms that could break down their feed during the usual process of feed absorption. Moreover, considerable effort has been devoted into diverse sustainable strategy that could be applied for prevention of the constant release of methane gas into the atmosphere. This chapter intends to present the process involved in the action methanogenesis by rumen microorganisms as well as provide some sustainable strategy that could prevent the release of methane into the atmosphere. The rumen microbial community is attracting interest as an alternative source of beneficial biogenetic resource for potential industrial applications. The current trends in the biotechnology are more focused on direct gene modification towards enhancing the bioactivity process and end use functionality. Furthermore, gene enrichment technique has enhanced the industrial application of rumen microbial enzymes as feed additives for non-ruminant animals.

Current world agriculture scientists face a wide spectrum of challenges including climate change, urbanization, and environmental issues: accumulation of insecticide, pesticide, decay in soil organic matter, and sustainable use of natural resources of ruminant excreta. The availability of methane in the atmosphere is presently around two-and-half times more than pre-industrial stages and is increasing gradually and such escalation has imperative implications for climate change. The actual approximations of methane emissions are subject to a high degree of uncertainty, but the recent estimate advocates that annual global methane emissions are around 570 million tonnes. Methane is known to be one of the most important greenhouse gases contributed to global warming by the livestock sector, which is formed by anaerobic fermentation in the different portions of the gut, and their concentration varies significantly among species. Methane is synthesized from certain types of microorganisms known as methanogens and the species conformation of methanogens is widely affected by the diet, geographical location, host, and gut portions.

We are honoured that leading scientists who have extensive, in-depth experience in soil biology took the time and efforts to develop the excellent chapters. The expert group of scientists uniquely suited to write these chapters and have firsthand knowledge of the methods and technique they have presented. We want to thank all the contributing authors for their diligence in bringing this volume. In all there are nineteen chapters covering most of the aspects.

We are grateful to the many people who helped to bring the volume to light. We wish to thank Dr. Sabine Schwarz from Springer, Heidelberg, Germany for generous assistance, positive critical comments, advise and patience in finalizing the volume. We are thankful to many faculty colleagues, students, and administrative staff for their constant support. Finally, we give specific thanks to our families—immediate and extended—for their kind support.

Noida, Uttar Pradesh, India Shubhangi Mahajan
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Contents

Chapter 1
Biotechnology of Rumen Microorganisms: Recent Advances

Charles Oluwaseun Adetunji, Olugbemi T. Olaniyan,
Osarenkhoe Omorefosa Osemwegie, Rashmi Dash, and Ajit Varma

Abstract The rumen of ruminant and non-ruminant animals has a pool of microorganisms (protozoa, bacteria, and fungi) that have biotechnological benefits as well as untapped potentials. These microorganisms are equipped with a wide range of enzyme-producing genes and enzymes which are found to be of interest in industrial applications. Suffices to say, rumen's microbial-produced enzymes are potentially explored to degrade toxins, plant cell wall polymers, synthetic polyesters, and different organic as well as synthetic substrates. Moreover, they are globally becoming an emerging resource for the process development of numerous eco-sustainable products. Therefore, this chapter explores the biotechnological role of rumen microorganisms, their characterization (biological and molecular), beneficial enzyme diversity and extraction techniques, and biodegradation values.

Keywords Biodegradation · Rumen · Rumen microbiome · Ruminant microbes

1.1 Introduction

Diverse microorganisms have been implicated in several biotechnological processes related to agriculture, medicine, drug development, aquaculture, and industries and more recently hypothesized to have the potential for carbon sequestration or

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_1

elimination as well as fixation (Zahed et al. 2021). These ubiquitous microorganisms, which are representative of all three domains (Eukarya, Archaea and Bacteria), are fundamental to the sustainability of planet earth due to their capacity to naturally drive eco-biological processes of biogeochemical cycles. Their adaptive versatility to climate change and genetic diversity makes them strong candidates to biotechnological experimentations (Singh et al. 2008). However, majority of the global microbial diversity and genetic resource pool are untapped for human application benefits. Even though humanity's focus has shifted to screening biogenetic resources for specific potentials of bioequivalent function(s) to synthetic chemical products because of their increasing toxicity risks, studies of their use for novel product generation or modulation in existing products using transgenic technology are not adequately translating to bioeconomic development (Cowan 2000). Moreover, it has been reported that more than 99% of the population of microorganisms in the environment are not still detected nor their potential economic values fully established (Sekiguchi 2006). This is further deepened by isolation, cultivation, and identification challenges of these diverse microorganisms. The emergence of several DNA-based molecular techniques such as 16S rRNA gene and 18S rRNA has however revolutionized their application as well as biotechnology. These techniques have improved the identification biogenetic species and the knowledge of microbiological taxa available in an environment. The knowledge of the numerous functional roles of various microorganisms in the delivery ecosystem services and genetic amenability in a changing environment or niches have also been advanced by these techniques (Streit and Schmitz 2004).

Microorganisms have been reported to dwell in the digestive tract of ruminant animals and colonize the abomasum, rumen, omasum, and reticulum where they facilitate digestibility of plant polysaccharides compared to the single-compartmented stomach of monogastric animals (Agarwal et al. 2015; Stevens and Hume 1998). These microorganisms are referred to as rumen microbiome, composed largely of bacteria and protozoa, majority of which are symbiotic (Hobson and Stewart 1997; Goel et al. 2015). Rumen microbiome have implicated numerous significant functions that are evident in the host immunity, physiology, pathology, and nutrition while they also enhance the host's capacity to regulate the nutrient absorption and breakdown of plant secondary compounds as well as methane production (Li 2015; John Wallace 2008; Jami et al. 2014). Furthermore, rumen ecosystem possesses numerous underutilized microbial resources that could potentially generate useful enzymes, particularly those associated with the digestion and breakdown of plant-based compounds (Morgavi et al. 2013; McCann et al. 2014; Krause et al. 2003). This chapter, therefore, reviews current advancements in the biotechnological application of rumen microorganisms for sustainable bioeconomic benefits on humanity.

1.2 Biotechniques Used in the Characterization of Rumen Microorganisms

There are numerous techniques that have been used for the characterization of gut microbial communities. Examples of such techniques include metagenomics, genomics, and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). These are applied to uncover the biological and structural diversities of rumen microbiome and their relative dynamics subject to change in rumen microenvironment (Morgavi et al. 2013; McAllister et al. 2006; Morgavi et al. 2013). Metagenomic technique is an important environmental microbiology tool used for the fast profiling of microorganisms in many ecosystems without a need for cultivation.

Interestingly, mega-genomics, environmental genomics, and community genomics have emerged as modification variant of metagenomics in the course of time (Chistoserdova 2009). Similarly, genes present in rumen microbial communities at either DNA or RNA levels (metagenomics) or aggregate collection of genomes may be subjected to sequencing-based characterization or meta-transcriptomics, while high-throughput screening of cloned expression libraries is used to derive rumen DNA for gene products of interest (functional metagenomics) (Li 2015). The application of functional metagenomics has been recognized as effective in identifying ecosystem's microorganisms and their role in the environment (Chistoserdova 2009). Typical examples of such investigation at a functional level relied on hydrogen metabolism and degradation of cellulose, while phylogenetic techniques have been also paramount for the detection of species novelty (Vieites et al. 2010). Functional screening technology has been implicated in the detection of novel enzyme domains in rumen materials (Ferrer et al. 2005). Despite this, there is a need for more knowledge on the ecology and functional dynamics of rumen microbiome as well as how their natural role can be adapted for their culturing. There is also limited information on the mechanisms used by these rumen microbes in breaking down plant polysaccharides relative to the host's rumen function (Wilson 2011).

1.3 Biotechnological and Industrial Relevance of Rumen Microbes

The rumen microbial community is attracting interest as an alternative source of beneficial biogenetic resource for potential industrial applications. The current trends in the biotechnology are more focused on direct gene modification toward enhancing the bioactivity process and end-use functionality. Furthermore, gene enrichment technique has enhanced the industrial application of rumen microbial enzymes as feed additives for non-ruminant animals.

Livestock feed production is boosted by the deployment of biotechnology to facilitate their digestibility, nutritional, health, and livestock's end-use benefits as well as toxin degradation. This trend has led to a reduction risk associated with livestock exposure to contaminated feeds and increased the demand for the production of more stable and effective enzymes that could degrade xylan, keratin, glycan, and cellulose. Furthermore, the influence of the rumen microbial enzyme source may also initiate anti-nutritional effect in the feed. The application of recombinant DNA technology has also led to a lot of improvement in the production of healthy livestock as well as the production of more cost-effective animal feed. Moreover, other relevant approaches that are involved in effective delivery and improvement of livestock include the secretion of digestive enzymes in the pancreas of transgenic animals, extraction of oleosin proteins from canola, and expression of seed proteins (Cheng et al. 1999).

1.4 Microbial-Derived Rumen Enzymes

Wang and McAllister (2002) reported that the microbial ecosystem in ruminant animals is responsible for the digestion of the lignin, phenolic, and polysaccharide compounds. Exogenous fibrolytic enzymes produced biotechnologically are utilized as feed additive for some monogastric animals. Through feed biotechnology, and exogenous fibrolytic enzyme additives, the development and digestion of livestock have experienced improvement in the past few decades. However, many factors, particularly enzyme source, type of enzyme (ferulic acid esterase, pectinases, proteases, cellulose, acetylesterase, xylanase, beta-glucanase), physicochemical nature of the diet, type of livestock, and mode of application, influence the process outcomes.

Santra and Karim (2002) reported that rumen microorganisms can digest different lignocellulosic feedstock while also recognizing the fact that associated enzymes can be manipulated to enhance digestive functions and optimize nutrition and productivity. This they noted may be by initiating an alteration of the rumen fermentation process using chemical additives, natural or genetically modified foreign microbes. Some of the advanced biotechnological manipulations of rumen's microbiome are also inclusive of interspecies trans-inoculation of rumen with microbes, probiotics of yeast and bacteria as feed composite, and other genetic manipulations of rumen enzymes.

Ribeiro et al. (2016) reported that rumen microorganisms can be mined for biotechnological manipulation into fibrolytic feed enzymes to enhance milk production, meat development, digestive process, and nutritional status of livestock through omics technology such as meta-transcriptomics and metagenomics. In ruminants, complex cellulose, plant-associated fungal mycotoxin, and cyanobacterium toxin digestion occurs through collective enzymatic actions of endoglucanase, xylanase, hemicellulase, and salivary cellulases. These enzymes have the capacity to digest plant toxins into specific products and secondary metabolites. Gordon and Phillips

(1998) corroborated this by noting that biotechnology affords cellular enzyme enhancement of the metabolic and biofunctions of xylanases, phenolic esterases, and cellulases.

1.5 Biotechnology Studies and Functions of Rumen Microorganisms

Studies acknowledged animal rumen as a fermentation and metabolic site and estimated to be about one-seventh of the total mass of the animal under relatively constant temperature. The salivary secretions of most ruminant animals possess buffering constituents that regulate heat, ammonia, methane, and volatile fatty acids toxic to microbial metabolism. Numerous products and intermediate compounds such as carbon dioxide, formate, ethanol, acetate, butyrate, lactate, and propionate are generated proportionately to the quantity of diet under digestion by the rumen microbes.

The degradation of polysaccharides has been reported to involve three stages: (1) attachment of microbes (protozoa and bacteria) to plant materials and subsequent degradation of plant carbohydrate polymers, (2) hydrolysis of liberated polymers into small saccharides by several extracellular enzymes, (3) intracellular fermentation process of the small saccharides and pectins into energy-intermediate products like phosphoenolpyruvate, ethanol, lactate, succinate, propionate, and pyruvate. The degradation process has been suggested to overlap into the glycolysis and pentose cycles. *Selenomonas ruminantium* is reported to produce one of the important enzymes involved in the degradation and fermentation process in ruminant animals.

Protein metabolism in rumen fermentation by protozoa and bacterial proteolysis involved the formation of ammonia and nitrogen which act as base for bacteria growth. This complex in fermentation was according to Russell and Hespell (1981) grouped into nutritional, physiological, biochemical, and biological events. Fanning (2016) reported that ruminant acidosis is an important activity in ruminant-fed diets and categorized forms based on pH. A pH range of 5.0–5.5 is called subacute ruminal acidosis, while the other pH range of less than 5.0 is referred to as the acute ruminal acidosis. The ruminal fluid pH is regulated mainly by salivary secretion, volatile fatty acids from the epithelium of ruminant, metabolic activity associated with the degradation of volatile fatty acids by microbes, and the clearance level of the ruminal fluid.

Coleman (1979) reported that rumen ciliate protozoa are an important factor in the metabolism of tropical feeds. The rumen protozoa were reported to influence blood flow, growth, and metabolism within the rumen cavity. Suffice to say, those protozoa play important roles in the host nutritional status through impacting the nitrogen and non-microbial nitrogen proportion. Protozoa is known to attach to plant particles and cause chemotaxis release of glucose which may be converted to starch and acetic, lactic, and butyric acids through ruminal fermentation. According to

Gupta et al. (2016), enhancing the nutritional status for improved rumen microbe's development is very important for calf development and growth and reduces susceptibility to diseases. Studies have revealed that the basic polymer in ruminant production process involving superorganisms is the lignocellulose which hydrolyzes to form the ruminant protein by saccharification (Morgavi et al. 2013). Many important enzymatic mechanisms and processes which influence the rumen microbiome can be positively optimized for animal growth and development by omics technology.

Conversely, Satyanagalakshmi et al. (2015) reported emerging research studies on ruminant methanogens resulting in the growing global concern about methane and other greenhouse gas emissions. However, ruminant methanogens can be controlled vaccines, dietary composition, analogs, fungal metabolites, and secondary metabolites. This aroused the attention of Okoruwa and Aidelomon (2020) who utilized lime peel and ginger powder for manipulation of rumen microbiome and rumen cavity fermentation process. Secondary metabolites such as iso-butyrate, iso-valerate, and valerate have the potential to interactively enhance the growth and development of sheep and rumen fermentation process with no significant adverse effects. This suggests that these compounds are significant in reducing the protozoa, methanogens, butyrate, and acetate. Furthermore, Castillo-González et al. (2014) reported a collaborative approach by numerous ruminal microorganisms within the necessary microenvironment of the rumen ecosystem and symbiosis with the host in advancing fermentation and digestion processes. The energy generated from the end products of fermentation process in the host animals is used by the microorganisms. Also, the nutritional status of the host is enhanced through the use of additives such as tallow, monensin, buffers, probiotics, and nitrogen compounds. This implies that the productivity of the host ruminant system increased with reduced energy loss.

Castillo-Lopez and Domínguez-Ordóñez (2019) observed that rumen contributes highly to microbial protein production which is estimated to be necessary for high nutritional status. These estimations were done through molecular biology techniques like high-throughput DNA sequencing and real-time PCR. Light microscopy and real-time polymerase chain reaction showed that the rumen of buffalo fed with different feeding systems like feedlot and grazing had diverse microbial species in the rumen-reticulum ecosystem (Franzolin and Wright 2016). Shakira et al. (2012) reported that innovative approach can be developed for rumen microbiome and changed subject to age, diet, antibiotic use, geographical location, host health, feeding scheme, and season. Using the classical culture-based techniques, like isolation, nutritional characterization, and enumeration by metagenomics, real-time PCR, and gene sequence analysis, the authors revealed that elevated microbial population is linked to the action of yeast in enhancing ruminant productivity and yield. Likewise, Daba et al. (2009) used different feed supplements to analyze the rumen microflora in fistulated animals. Many rumen animals were observed to lack enzymes to metabolize fibrous feeds and thus were affected by the numerous substrates used for the study. Also, it was discovered that feed supplements which are protein based (tree Lucerne and cottonseed cake) and energy fiber based (molasses and wheat bran) could be degraded by dynamic rumen microbial diversity for enhanced animal products and quality.

Wang et al. (2016) showed that diet containing purified starch generated from high or normal amylose maize grains can influence rumen blood profile and fermentation process in goats. The authors demonstrated that high amylose starch supplements showed multiple beneficial effects in animals. Sha et al. (2020) characterized and analyzed the functions of rumen microbial population in cattle yak; thus, environmental and physiological adaptations were analyzed through metagenomic analysis. They discovered that several microorganisms are involved in lignocellulose digestion which include *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Prevotella ruminicola*, *Fibrobacter succinogenes*, and *F. succinogenes*.

Bekele et al. (2021) utilized functional metagenomics to analyze the rumen microenvironment and the role in maintaining essential physiological microbiome, nutritional status, and host immunity, thereby reducing pathological conditions. In their study, they were able to unravel the potential of microbial rumen processes involving enzymes like carbohydrate-active enzymes through function-based and sequence-based metagenomics.

Getabalew and Alemneh (2019) revealed that biotechnology is currently being utilized to enhance livestock feed and improve the physiology rumen microbiome. They reported that forage usage in feeds, feed additives, and rumen microbiome manipulations can grossly improve the overall feeding pattern of livestock, thus enhancing the human health and food security through quality assurance strategy, public health safety, and nutritive value. Studies have shown that biotechnology can improve many aspects of animal feeds and thus enhance their nutritive value. For instance, through biotechnology, gut microbiomes can be enhanced thus improving metabolism, growth, and overall health of livestock. Biotechnology products like enzymes, antibiotics, prebiotics, and probiotics can be used. Additionally, the use of new strains or species of microbes or genetically modified organisms to effect digestion of fibrous constituents, degradation of toxins, lignin of forage, production of amino acids, and reduction in formation of methane is biotechnologically driven. It has been shown that microorganisms particularly protozoa play a key role in maintaining the concentration of fermented products like ammonia, methane, lactate, butyrate, and propionate (Patel and Ambalam 2018).

Faixová and Faix (2002) reported that many ions can influence the activity of gut rumen enzymes positively thus enhancing their metabolism in these animals. Metallic ions such as cadmium, magnesium, and copper can act on rumen fluid enzymes such as alanine aminotransferase, urease, aspartate aminotransferase, glutamate dehydrogenase, and gamma glutamyltransferase.

Krause et al. (2003) reported the use of genomics, microbiology, high-throughput sequencing, and ecological tools and manipulations in enhancing fiber degradation by rumen microbiota fibrolytic enzymes and exogenous enzymes through fermentation process. Some of the exogenous enzymes can be found in dietary forage known to improve rumen fermentation process, growth performance, and bacteria activity as shown by Liu et al. (2019). These end products of fermentation are utilized for the production of glucose, lipid, and protein.

Mamuad et al. (2019) reported the utilization of live *Enterococcus faecium* supplementation in enhancing rumen fermentation and microbial community composition, thus influencing productivity and health. The authors revealed that through the supplements, there was significant increase in total volatile fatty acids and propionate levels with reduction in methane production.

1.6 Conclusion and Recommendation

This review provides information on the application of rumen microorganisms with bias for their biotechnological values and techniques deployed for their modification, manipulation, and functionality improvement. Harnessing them for the production of relevant enzymes that are potentially valuable for industrial applications and preparation of animal feeds for development of livestock trade are also mentioned. Techniques involved in the characterization of rumen microbiome and next-generational sequencing will also go a long way toward the detection of some new and effective strains for a range of human benefits. The application of metabolomics and proteomics should also be introduced into microbiology of rumen so that more special biological active constituents capable of improving human well-being, global food, and safety security can be identified (Adetunji and Anani 2021a, b; Okeke et al. 2021; Adetunji et al. 2021a, b, c, d; Adetunji et al. 2020; Olaniyan and Adetunji 2021). Therefore, this has affirmed the bioeconomic values of rumen ecosystem and supports the view that it is now attracting interest for the production of novel products such as antibiotics, detoxificant, and enzymes.

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Chapter 2
The Process of Methanogenesis by Rumen Microorganisms: State of Art

Charles Oluwaseun Adetunji, Olugbemi T. Olaniyan, Rashmi Dash, and Ajit Varma

Abstract It has been observed that rumen microorganisms are involved in the biodegradation and fermentation of cellulose to different monomers and other compounds. Also, the rumen methanogenesis and fermentation process are affected by many factors such as nutritional composition, secondary metabolites, degradability, and season. There are many stages that are involved in the action of different bacteria like hydrolyzing, acetogenic, digestive, homoacetogenic, methanogenic, and sulfate-reducing (VI) bacteria which can decompose organic constituents such as methane to generate biogas. Therefore, this chapter intends to provide some detailed information on the process involved in the methanogenesis by rumen microorganisms. Also, relevant information on the modes of action involved in the process of methanogenesis by rumen microorganisms was provided, while different types of activities performed during methanogenesis by rumen microorganisms were also highlighted.

Keywords Methanogenesis · Fermentation process · Rumen · Acetogenic · Digestive · Homoacetogenic · Methanogenic and sulfate-reducing bacteria

2.1 Introduction

The production of methane most especially through the action of enteric fermentation has been recognized for its action that led to the buildup of greenhouse gases in the surrounding, together with the significant role they play in the loss of fed energy

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_2

for the animal. Methane is generally generated in the hindgut of animals and in their rumen through the action of *Archaea* recognized as methanogens which could be classified to phylum Euryarchaeota (Kumar et al. 2009).

It has been revealed that ruminants retain the competence to produce the greatest level of methane through the action of methanogens microorganisms that could break down their feed during the usual process of feed absorption. Moreover, considerable effort has been devoted into diverse sustainable strategy that could be applied for prevention of the constant release of methane gas into the atmosphere (Shibata and Terada 2010). Therefore, this chapter intends to present the process involved in methanogenesis by rumen microorganisms as well as provide some sustainable strategies that could prevent the release of methane into the atmosphere.

2.2 Mechanism of Action of Methanogen Microorganism in Ruminant Production

Ruminant production has been under enhanced public inspection in terms of the significant of ruminant animal because they possess the capability to generate greenhouse gas methane. Methanogenesis is normally carried out through the action of methanogenic archaea, a unique group of microorganisms that are available in anaerobic environments most especially in the rumen. The methanogens possess the capability to apply H_2 and CO_2 as substrates so as to generate methane which confirms that they perform a significant role in the functional niche in the ecosystem. Furthermore, together with the methanogens, other microorganisms possess the capability to impact the level of methane fabrication because they are involved in hydrogen (H_2) metabolism and they could influence several other members of the microbiota and most especially the methanogens. Also, there are some associations that exist between microbes and methanogenesis as well as some functional groups that perform the function of reducing the process involved in the methane emissions. It has also been affirmed that dihydrogen ("H_2" from this point on) has been identified as one of the major element that regulates the process involved in the manufacturing of methane in the rumen.

Furthermore, it has been discovered that protozoan occupies a protuberant position which is enhanced by their relationship with methanogens that encourage the movement of H_2 with the system. Also, some microorganisms that could emit methane have a stronger association between protozoan numbers. This might be linked to the fact that the presence these group if microorganisms are not required in the rumen to function but the presence of protozoan could play a crucial role in the prevention of methane. Their relation has been associated with the generation of H_2 as a result of breaking down of plant containing fibrous materials, but not all members associated with rumen fibrolytic community could generate H_2.

It has also been affirmed that enhancement of the non-H_2-producing fibrolytic microorganisms could reduce the generation of methane without having any influence on the process involved in the degradability of forage. Also, there is presence of different pathways that utilize electron acceptors when compared to utilize CO_2 to oxidize H_2 in the rumen. It has been observed that these types of bacteria-specific metabolism could inhabit a specific ecological niche without affecting the other members of the microbiota. But there is capability that their number will rise provided the required number of electron acceptor is available in the diet.

Furthermore, it has been documented that nitrates represent another alternative electron sinks that could enhance the development of some bacteria that could compete most especially with methanogens. Moreover, nitrite has been identified as an intermediate product, but its application has not been fully established but fully applied in animal. It has been observed that nitrate does not build up, while nitrate supplementation could be another dietary condition that needs to be investigated. Therefore, the presence of methanogens in the rumen together with other microorganisms will give a better insight on the pathways that could lead to the prevention of the constant release of methane from ruminant animals which are responsible for greenhouse effect (Morgavi et al. 2010).

2.3 Process of Methanogenesis by Rumen Microorganisms

Marlina et al. (2018) reported that microorganisms are responsible for the production of methanogenic organic materials. The authors showed that anaerobic microbes, fungi, endoparasites, and protozoa are enlisted as major sources of methane production. The fluid obtained present in the rumen was obtained from beef cattle waste and dairy cattle waste and was later subjected gas chromatography analysis. Moreover, it was observed that methane production was generated from livestock waste by methanogenic microbes.

Morgavi et al. (2010) revealed that the major producers of the greenhouse gas methane are the ruminant animals. The authors revealed that methanogenic archaea generate methane and methanogens in an anaerobic environment through methanogenesis using CO_2 and $H_{2.}$ It was demonstrated that protozoa are particularly indicated to display a potential role in methanogensis; thus these organisms may be a potential target in the regulation of methane production by ruminant animals. The authors equally highlighted the role of fibrolytic organisms in the mitigation of methane production without decreasing the function of forage degradability. Methane production mechanisms involve the fermentation process, but the manipulation of the rumen microbial contents has been demonstrated to have a great effect in reducing this production.

Studies have revealed that ruminant animals have potential to generate serious economic value and impact through the production of methane used as by-product in fermentation processes. Accumulation of this methane has been seriously considered as a major problem in climate change and global warming through methanogenic archaea which is estimated to be about 15% of the total methane discharges in the atmosphere. The methanogens responsible for the production of methane can be cultured through PCR-based techniques; thus, the hydrogen from rumen microbiota was reduced to propionate through fumarate and lactate (Mitsumori and Sun 2008). In an attempt to reduce the production and emission of methane, different approaches have been utilized through microbial manipulations like antibiotics, chemicals agents, natural products like oils, plant extracts, and fatty acids. Several studies have indicated that the use of vaccines can be utilized for the control of methanogenic microbes which showed that these drugs do not decrease the ingestion of feed or digestibility but can improve propionates (Kobayashi 2010).

Widjaja et al. (2017) reported that microbe present in the rumen fluid and cow dung could produce environmentally friendly energy from coffee pulp like tannin, free phenol, and caffeine. Kataria (2015) stated that utilization of saponins, fats, oils, and tannins, probiotics, bacteriocins and ionophores, prebiotics, vaccination and inoculation of exogenous bacterial strains could help in the control of methane production.

Teklebrhan et al. (2018) reported that diverse rumen microbiota is responsible for ruminal methanogens with different co-enzymes for the production of biogenic methane which has been reported to cause climate change. The authors showed that the methane production varies with geographical location, animal age, seasonal variation, feed composition, diet, section in gut, animal species, and management. The metabolic pathway and substrate fermentation process utilize acetate, methyl group compounds, and CO_2 in the gut microenvironment (Abecia et al. 2012).

Studies have revealed that different bacteria like hydrolyzing, acetogenic, digestive, homoacetogenic, methanogenic, and sulfate-reducing (VI) bacteria can decompose organic constituents such as methane to generate biogas. Several studies have demonstrated that different molecular biology techniques such as meta-transcriptomic, co-culture-based, and comparative genomic methods can be utilized for thorough understanding of ruminant microbial fermentation, enzyme physiology, and digestive process. It has been revealed that rumen efficiency is determined by animal selection, methanogenesis inhibitors, and dietary supplementation.

Hook et al. (2010) reported that microorganisms referred to as methanogens are methane-producing organisms in the rumen microenvironment in cattle or sheep. The authors showed that geographical location, diets like lipids, monensin, plant constituents, organic acids, or vaccines can be utilized to modulate the host methanogenesis. Behlke (2007) reported that ruminal methanogens produce methane which can be manipulated through diets using nitrofuran derivative of para-aminobenzoate, bioactive molecules derived from extract Yucca schidigera, 2-bromoethansulfonate, corn-based diets, and brome hay-based diets. Studies have revealed that methane-producing archaea can be found in the environment, marine sediment, digestive tracts of humans and animals, terrestrial environments subsurface, insects, and marine sediment (Buan 2018).

Ahring et al. (2018) reported that cellulolytic microbes are involved in the biodegradation and fermentation of cellulose to different monomers and other compounds. The authors demonstrated that 2-bromoethanesulfonate can selectively be utilized for inhibition of methanogens and reduce total volatile fatty acid productivity using endoglucanase assay. Danielsson et al. (2017) reported that the quality and yield of methane vary in terms of specie type and feeding conditions. In their study, qPCR was utilized in the analysis of the 16S rRNA gene of rumen of methanogenic groups while some other products such as propionate, volatile fatty acids, and butyrate was liberated during the fermentation. Also, no significant difference was recorded for the cows concerning milk digestibility and milk production.

Kim et al. (2013) studied the role of plant extracts *Ginkgo* leaves and pine needles through in vitro fermentation in addition to rumen methanogens for decreasing the emission of methane gas into the environment. Using real-time PCR, *Fibrobacter succinogenes* and four groups of methanogens were evaluated, and gas profiling revealed hydrogen, methane, and carbon dioxide. It was demonstrated that the plant extract reduced the emission of methane and the presence of volatile fatty acids and methanogens as compared to the control group, thus suggesting that they could be utilized as additive in ruminants.

Zhang et al. (2020) reported that nitroethane, 2-nitro-1-propanol, and 2-nitroethanol were able to cause inhibitory action against rumen fermentation and methanogens, thus reducing the overall production of methane. It was reported that these nitro compounds were able to cause inhibition through reduction in co-enzyme F420 and F430 and mcrA gene expression. Wilkie et al. (1986) made a report on micronutrients like cobalt, nickel, selenium, molybdenum, and sulfate which significantly increased methane generation but reduced volatile fatty acids in mesophilic anaerobic digestion of napier grass.

Cieslak et al. (2013) showed that certain plant constituents can block the activities of rumen methanogens. In their study, they revealed that bioactive components popularly known as phytochemicals like tannins, saponins, essential oils, and flavonoids can potentially modulate the process of fermentation and digestion in rumen. This signifies that they could be utilized as additives in animal feeds to mitigate methanogenesis and their toxic effects. Generally, the rumen methanogenesis and fermentation process in rumen are influenced by countless factors such as nutritional, secondary metabolites, degradability, and season. Essential oil has been identified as a modifier of the rumen digestion and fermentation process due to their significant antimicrobial activity.

Kurniawati et al. (2018) reported that red ginger has tremendous amount of essential oil which was analyzed against the fermentation parameters, pH, volatile fatty acid concentration, ammonium, microbial number, and microbial protein. From their experiment, it was discovered that red ginger essential oil significantly reduced ammonium level and protein degradation in rumen. Patra and Yu (2013) investigated the role of coconut oil and fish oil on rumen fermentation and digestion process influencing methane generation in in vitro rumen cultures from lactating cow. From their results, it was shown that both oil extracts were able to reduce methane production, but there is disparity in terms of microbial population, mode of action, and diversity. Thus, the authors suggested the combination of both oils to reduce the adverse effects and increase performance.

2.4 Conclusion and Recommendation

This chapter has explained numerous processes entailed in the methanogenesis by rumen microorganisms. Various molecular biology techniques such as meta-transcriptomic, co-culture-based, and comparative genomic methods that could provide a better insight on the application of ruminant microbial fermentation, enzyme physiology, and digestive process were also highlighted. Moreover, the application of next-generation sequencing will provide a better understanding of some novel strains that could be a source of different industrially relevant product derived from rumen microorganism (Adetunji and Anani 2021a, b; Okeke et al. 2021; Adetunji et al. 2021a, b, c, d; Adetunji et al. 2020; Olaniyan and Adetunji 2021). The application of metabolomics can also provide more facts on appropriate metabolites that could be applied in the generation of useful product with higher relevance in the livestock industry most especially those produced during the process of methanogenesis by rumen microorganisms.

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Chapter 3
Rumen Methanogenesis and Mitigation Strategies

Kaushalendra Kumar and Vinod Kumar Paswan

Abstract The availability of methane in the atmosphere is presently around two-and-half times more than pre-industrial stages and is increasing gradually, and such escalation has imperative implications for climate change. The actual approximations of methane emissions are subject to a high degree of uncertainty, but the recent estimate advocates that annual global methane emissions are around 570 million tonnes. Methane is known to be one of the most important greenhouse gases contributing to global warming by the livestock sector, which is formed by anaerobic fermentation in the different portions of the gut, and its concentration varies significantly among species. Methane is synthesized from certain types of microorganisms known as methanogens, and the species conformation of methanogens are widely affected by the diet, geographical location, host, and gut portions. The three major orders of methanogens in gut environments are Methanomicrobiales, Methanobacteriales and Methanosarcinales and normally present in less numbers (below 3% of total microbes). The key changes in archaeal activity among various gastrointestinal parts and animal species are usually the fermentation of substrate and metabolism to complete the anaerobic process of plant material. Generally, three key substrates are used by archaea such as CO_2, acetate and methyl group-containing compounds. Therefore, the present chapter will describe the metabolic pathways and methanogens involved in enteric CH_4 synthesis as well as their mitigation strategies to combat the challenges of global warming effect for sustainability.

Keywords Global warming Greenhouse gas · Livestock-associated environmental pollution · Methane emission · Ruminal archaea

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_3

3.1 Introduction

In present scenario, global warming became one of the important challenges for livelihood around the globe. The recent approximations specify that global temperature will upsurge between 1 °C and 6 °C during the twenty-first century (IPCC 2015), due to accumulation of greenhouse gases in the atmosphere. Enteric methane emission from ruminants causes loss of feed energy, also contributing to the global warming effect. Methane is synthesized as one of the hydrogen sink products, which is inevitable for competent succession of anaerobic microbial fermentation in ruminants. However, methane pays nearly 16% of the greenhouse gas emissions, which also has 21 times greater global warming potential (GWP) than that of carbon dioxide (Scheehle and Kruger 2006). Nearly 36% of the total methane released in environment are from natural sources like wetlands, oceans, termites and wild ruminants, whereas the remaining 64% contributed from anthropogenic sources like fossil-fuel use, livestock farming, landfill and paddy field (Bousquet et al. 2006). Therefore, such type of human activities encouraged two-fold increase in the methane volume over the past 150 years, and such emissions have burdened the natural sinks on earth (IPCC 2015). After fossil-fuel use, enteric methane gas production is the second-most important source which represents 27% of the anthropogenic methane emissions (Bousquet et al. 2006). Herrero et al. (2016) revealed that non-carbon dioxide releases from livestock range from 2 to 3.6 Gt CO_2eq, with the key contributor being enteric fermentation from ruminants, where cattle are the chief source of emissions as 65–77% of the total volume (FAO 2013; Herrero et al. 2013).

Moreover, livestock in low- and middle-income countries contributes 70% of the emissions from ruminants and 53% from monogastric animals, and the numbers are likely to rise as the demand for livestock products is continuously increasing in these countries. However, productions and intensities are heterogeneous all over the world, and mixed crop-livestock systems account for 58% of livestock emissions, whereas grazing systems contribute 19% of sectoral emissions and industrial systems, conquered by monogastric production contributing to the remainder (23%). It is important to mention that livestock sector has reduced emission intensities by 60% since 1961 (Davis et al. 2015), but by-products like red meat are still the utmost inefficient in terms of releases per kg of protein produced by a factor of five or more in comparison to milk or pork, eggs and all crop products.

Various efforts have been made to diminish methane emission, mainly through dietary manipulation of rumen ecosystem, by the application of different chemicals, antibiotics, natural products like oils, fatty acids, polyphenols and plant extracts as well as development of vaccines against methanogens. Ionophore anibiotics are widely used due to their efficacy and price reasonability, while application of various natural substances is more attractive due to the health concerns in respect of antibiotics. Moreover, enteric methane emission causes considerable amount of feed energy which is equivalent to 2–12% of the gross energy of animal feed (Johnson and Johnson 1995). There are some implications of these approaches in methane mitigation, and an introduction to antibiotic-alternative natural materials and novel tactics is provided. Therefore, the present chapter aims to provide an inclusive view to understand the intricacy of the methanogenesis process in the gut and methane-producing archaea and thus to develop profitable methane-mitigation strategies for sustainability of livelihood.

3.2 Biochemical and Microbial Background Associated with Methanogenesis

Methanogenesis is a form of anaerobic respiration that implies carbon as the terminal electron acceptor, resulting in the production of methane, where carbon is obtained from a minor number of low-molecular-weight organic compounds, such as carbon dioxide, acetate, formate, methanol, methylamines, dimethyl sulfide and methanethiol. Microbes capable of producing methane are known as methanogens and those identified only from the domain Archaea, which is a group that is phylogenetically different from eukaryotes and bacteria. Generally, 250 litres of methane gas is liberated by a cow per day. According to Hedderich and Whitman (2006), the biochemistry of methanogenesis is a composite process which requires unique coenzymes and membrane-bound enzyme complexes, and major organic compounds like carbohydrates and volatile fatty acids are not the substrates for archaea which have been dealt by other microbes (bacteria, protozoa or fungi) prior to their use by methanogens; thus, most of the existing energy is being used by non-methanogenic organisms available in the gut. Methane production denotes a significant metabolic sink for hydrogen that would otherwise gather in the rumen creating an uncomplimentary environment for fermentative digestion processes (Morgavi et al. 2010). However, hydrogen itself does not accumulate due to methanogen activity; instead, methanogens take part in inter-species hydrogen transfer and dispose off the reducing equivalents from other metabolic processes (McAllister and Newbold 2008).

A methanogenesis pathway is presented in a simple illustration (Fig. 3.1), which includes the convergence of pathways known to occur in a *Methanosarcina barkeri*. According to Lambie et al. (2015), methanogens are categorized on the basis of their metabolic pathways such as hydrogenotrophic, acetoclastic and methylotrophic which can produce methane in the rumen by *Methanosarcina barkeri*. In spite of their inadequate number of substrates, methanogens are highly diverse in nature, and generally, their classification includes five orders (Methanobacteriales, Methanomicrobiales, Methanosarcinales, Methanococcales and Methanopyrales), which vary in more than 82% of their 16S rRNA sequence uniqueness, whereas recently, some more orders (Methanocellales and Methanomassiliicoccales) have been listed. Moreover, these orders have similar identity like capacity to fluoresce blue-green, due to presence of the coenzyme F420 (Ashby et al. 2001) or absence of

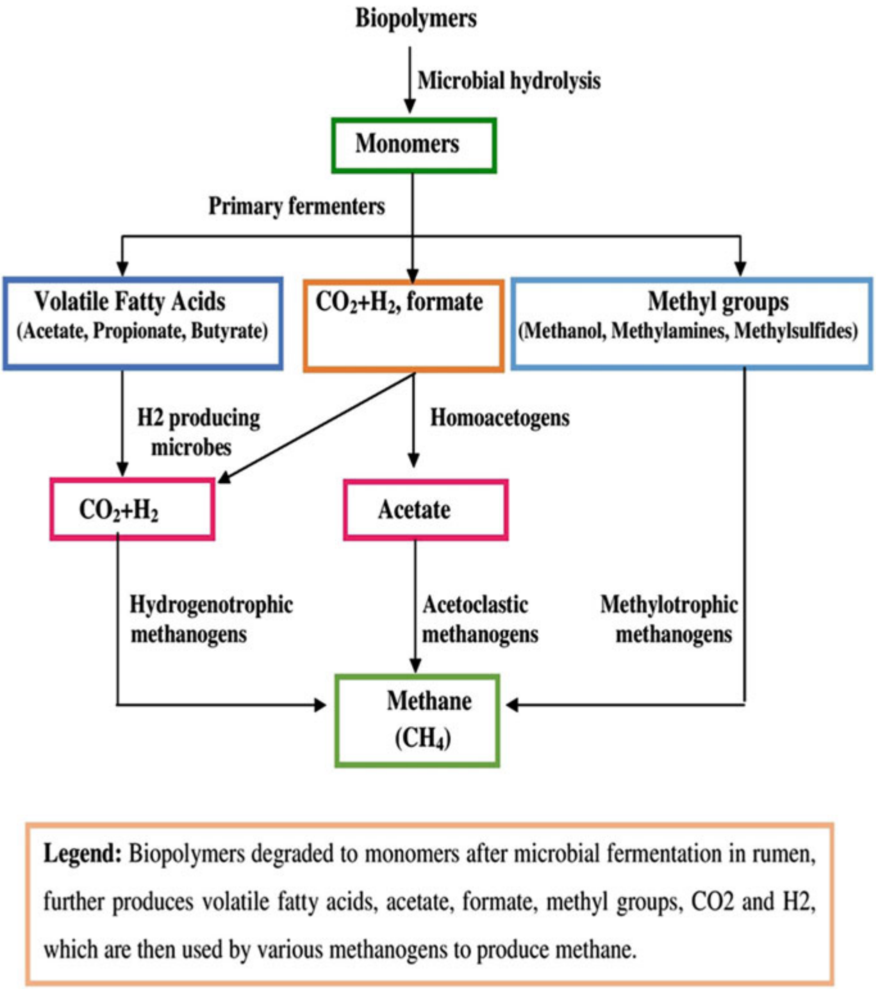


Fig. 3.1 Flow chart of methanogenesis during anaerobic fermentation of biopolymer

peptidoglycan in the cell wall, but it may also change on various biological aspects related to the structure of the cell wall, core lipids or substrates used.

Methanobacteriales mostly uses carbon dioxide and hydrogen (hydrogenotrophic in nature), but several species under this order can also use formate, carbon monoxide or secondary alcohols as electron donors. According to Hook et al. (2010), the most genera are rod shaped which form filaments, and foremost genera of interest in the gut methanogenesis within this order are *Methanobacterium*, *Methanobrevibacter* and *Methanosphaera*. Moreover, *Methanomicrobiales* order generally uses carbon dioxide and hydrogen, whereas major species can use formate, and few genera can also use secondary alcohols as electron donors. Liu and Whitman (2006) emphasized that the genera in this order differ in shape from cocci to rods and most species having protein cell walls, whereas some have glycoproteins. Out of the three families of this order, only one (Methanomicrobiaceae) is of interest in the gut methanogenesis.

Methanosarcinales order are the only methanogens with cytochromes (carries membrane-bound electron) that play significant role in the oxidation of methyl groups to carbon dioxide. Therefore, the species of this order have the comprehensive substrate range, having a methyl group (methylotrophs), acetate (acetoclastic), carbon dioxide and hydrogen (hydrogenotrophic), whereas they are non-motile and varying in structure from cocci to sheathed rods. The major species of this order have protein cell walls and cellular lipids having archaeol, hydroxyarchaeol and caldarchaeol. The important genera of interest of this order in gut methanogenesis are *Methanosaeta* which uses solely acetate, due to the presence of a high-affinity adenosine monophosphate. Moreover, *Methanosarcina* favours methanol and methyl amine to acetate, because its low-affinity acetate kinase (Jetten et al. 1992) and microbes of this genera are chiefly pertinent on the methanogenesis from manure.

Methanococcales have been isolated from marine habitats, which have no significance in gut methanogenesis. However, it produces methane by using carbon dioxide, formate and hydrogen as electron donors. The cell wall of this genera has S-layer proteins and is lacking in glycoproteins and carbohydrates and small cocci and motile in nature due to the presence of flagella. According to Liu and Whitman (2006), Methanopyrales order signified as single hydrogenotrophic species, namely, *Methanopyrus kandleri*, which is motile in nature, rod-shaped, its cell wall comprises pseudomurein and lipids contain archaeol, that inhabits only in marine ecosystems.

The newly described order of methanogens, namely, Methanocellales, was predominantly identified in rice-field soil (Sakai et al. 2008). Yuan et al. (2009) and Angel et al. (2011) revealed that they are unique among methanogens in their tolerance to oxygen stress and their adaptation to low-hydrogen partial pressures (Sakai et al. 2009). According to Tajima et al. (2001), another order, namely, Methanomassiliicoccales, is phylogenetically dissimilar from all other orders of methanogens and associated with non-methanogenic archaea like Thermoplasmatales. Janssen and Kirs (2008) reported that it creates one of the three principal archaeal lineages in the rumen, and in few ruminant species, it implies half or more of the methanogens (Wright et al. 2007). Moreover, Borrel et al. (2014) indicated that this order is largely distributed, and not limited to the gut of animals, although it is also recovered in rice paddy fields, natural wetlands or freshwater sediments.

The ruminant species have multifaceted microbial diversity which includes 10^{10}–10^{11} bacteria, 10^{8}–10^{9} methanogens, 10^{6} ciliate protozoa and 10^{6} fungi per millilitre of rumen fluid. The population of methanogens is greatly affected by the nature of diet, level and frequency of feeding. The rumen methanogens exploit reducing equivalents, produced by fermentative hydrogen-producing bacteria, anaerobic fungi and ciliate protozoa. However, ciliate protozoa are the only microbes for which such interaction can be microscopically demonstrated (Vogels et al. 1980), and there is a symbiotic relation between methanogens and ciliates, which may generate up to 37% of rumen methane emission (Finlay et al. 1994).

3.3 Factors Affecting Methanogenesis in Livestock

3.3.1 Topographical Distribution of Livestock Species

The archaeal diversity can be affected by inter-animal variations, diet, region and sampling process (Wright et al. 2007; Jeyanathan et al. 2011). According to Wright et al. (2007), both diet and geography of the host animals may perform significant role in changing the methanogen population. Henderson et al. (2015) opined that the *Methanobrevibacter gottschalkii* and *M. ruminantium* species, in most of the samples collected, accounting for 74% of all rumen archaea. However, *Methanomicrobium* spp. reported as an ample (>5%) methanogen in Asiatic region, but it is not universally prevalent.

3.3.2 Variation in Diets

Types and composition of diet impact methane synthesis in ruminants, because rumen fermentation depends on various nutrients like carbohydrate, protein, fat and minerals provided through ration (Moss 1994). Thus, quality of the diet exerts strong effect on the activity of the rumen microbiota and thus synthesis of methane impacted. Enteric methane emission intended to decline with a high protein ration, and vice versa happens when diet is rich in fibre (Kurihara et al. 1997). Moreover, highly digestible forages stay in the rumen for a shorter period, because of high passage rate, whereas forages having poor digestibility stand for longer period in the foregut, which lead to higher methane emissions. Ruminants offered with leguminous forages are testified to yield less methane from those feeding on grasses, which promote a more intake and higher production (Ramirez-Restrepo and Barry 2005).

3.3.3 Types of Animal Species

In ruminant animals, rumen is fermentation vat, where microbial breakdown of substrates takes place. However, hindgut fermentation in monogastric species varies from rumen fermentation, with a significantly lower methane production (Table 3.1). Methane synthesis and reductive acetogenesis may occur simultaneously in the hindgut of animals. The sulphate-reducing bacteria have a more substrate attraction for hydrogen than do methanogens; hence, methane synthesis occurs only in the absence of or under limiting sulphate scenario (Lovley et al. 1982). Furthermore, the

Table 3.1 Enteric methane emissions, common methanogens and probable pathways of methanogenesis in different livestock species

Host species	CH_4 produced in L/kg DMI (L/kg BW in parentheses)	Most abundant microorganisms	Most probable pathway	Reference
Large ruminants (cattle, bison, buffalo)	26–38 (0.56–0.76)	*Methanobrevibacter gottschalkii Methanobrevibacter millerae Methanobrevibacter smithii Methanobrevibacter thaueri Methanobrevibacter ruminantium Methanobrevibacter olleyae Methanosphaera stadtmanae Thermoplasmata*	Use H_2 to reduce CO_2 to CH_4 or reduce methyl groups derived from methanol or methylamines	Patra et al. (2017) Seedorf et al. (2015) Kelly et al. (2016) Henderson et al. (2015)
Small ruminants (sheep, goat, deer)	21–32 (0.40–0.71)	*Methanobrevibacter gottschalkii Methanobrevibacter millerae Methanobrevibacter smithii Methanobrevibacter thaueri Methanobrevibacter ruminantium Methanobrevibacter olleyae Methanosphaera stadtmanae Thermoplasmata*	Use H_2 to reduce CO_2 to CH_4 or reduce methyl groups derived from methanol or methylamines	Patra et al. (2017) Seedorf et al. (2015) Kelly et al. (2016) Henderson et al. (2015)
Camelids	16–24 (0.21–0.33)	*Methanobrevibacter millerae* *Methanobrevibacter ruminantium*	Use H_2 to reduce CO_2 to CH_4	Dittmann et al. (2014) St-Pierre and Wright (2013)
Pigs	2.3 (0.04–0.08)	*Methanobrevibacter ruminantium* *Methanobrevibacter wolinii* *Methanosphaera stadtmanae*	Use H_2 to reduce CO_2 to CH_4	Cao et al. (2016) Gong et al. (2018) Jensen (1996)
Rabbits	2.93 (0.13)	*Methanobrevibacter smithii*	Use H_2 to reduce CO_2 to CH_4	Franz et al. (2011)
Horses	6.1 (0.11–0.15)	*Methanocorpusculum labreanum Methanobrevibacter smithii Methanobrevibacter gottschalkii*	Use H_2 to reduce CO_2 to CH_4	Crutzen et al. (1986) Jensen (1996) Lwin and Matsui (2014)
Humans	0.07–6.67 (0.0006–0.06)	*Methanobrevibacter smithii Methanosphaera stadtmaniae*	Use H_2 to reduce: (1) CO_2 to CH_4 (2) methanol to CH_4	Gaci et al. (2014) Crutzen et al. (1986) Sahakian et al. (2010)
Macropods	4.9–11.24 (0.08–0.14)	*Methanobrevibacter gottschalkii Methanosphaera stadtmanae*	Use H_2 to reduce: (1) CO_2 to CH_4 (2) methanol to CH_4	Madsen and Bertelsen (2012) Evans et al. (2009) Klieve et al. (2012)

BW, body weight; CH_4, methane; CO_2, carbon dioxide; DMI, dry-matter intake; H_2, hydrogen
Source: Adapted from Fuente et al. (2019)

acetogens become active only when both sulphate-reducing bacteria and methanogens are less competitive to hydrogen uptake and acetogenesis, which appears to be significant in the caecum and proximal colon of pigs; the change in pH is a significant factor to control the rate of hydrogen uptake (Gibson et al. 1990).

3.3.4 Environmental Factors

Environmental temperature is also one of the crucial factors that regulate methane production, because intake and digestibility of feed vary with ambient temperature. According to Mbanzamihigo et al. (2002), there was an increase in enteric methane emissions during late summer (August–September) compared to early summer (June–July) in the Northern Hemisphere. Likewise, an experiment conducted in young wethers grazing, a moist hilly island pasture, a perennial rye grass/white clover dominant pasture and a late summer season pasture found higher methane yield in wethers grazing late summer season pastures, which is attributed to the quality deterioration of the pastures during the summer season (Ulyatt et al. 2005); thus, it has indirect effect of raised ambient temperature on the methane production through altered pasture characteristics.

3.4 Hydrogen Sink in the Rumen

The anaerobic fermentation mainly takes place in rumen, where numerous and dense microbial inhabitants have interdependent associations, in which various metabolites are exchanged, which boost or compensate each other’s growth, a process which is termed ‘cross feeding’ (Wolin et al. 1997). Methane synthesis appears as one such cross feeding between hydrogen-producing microbes and hydrogen-consuming methanogens. Moreover, the hydrogen-producing microbes comprise fibrolytic fungi and bacteria, and their co-association with archaea permits effective purging of hydrogen, which eases constant fibre degradation.

When methane mitigation strategies attempted in animals, then it is essential to consider alternative hydrogen sinks for methanogenesis, the detailed pathway of hydrogen regulation exist in the rumen elaborated (Fig. 3.2). According to Mitsumori and Sun (2008), methanogenesis is the prime pathway followed by propionate synthesis (fumarate reduction); however, reductive acetogenesis, sulphate reduction, nitrate and nitrite reduction and biohydrogenation of unsaturated fatty acid made quite a minor role in hydrogen consumption in the rumen. Therefore, strategy for enhancing of propionate production in gut can reduce methane production efficiently; else, rumen fermentation could be disturbed by hydrogen accumulation due to lack of hydrogen elimination by methanogenesis. However, the mode of these variations fluctuated depending on several manipulation processes, whether

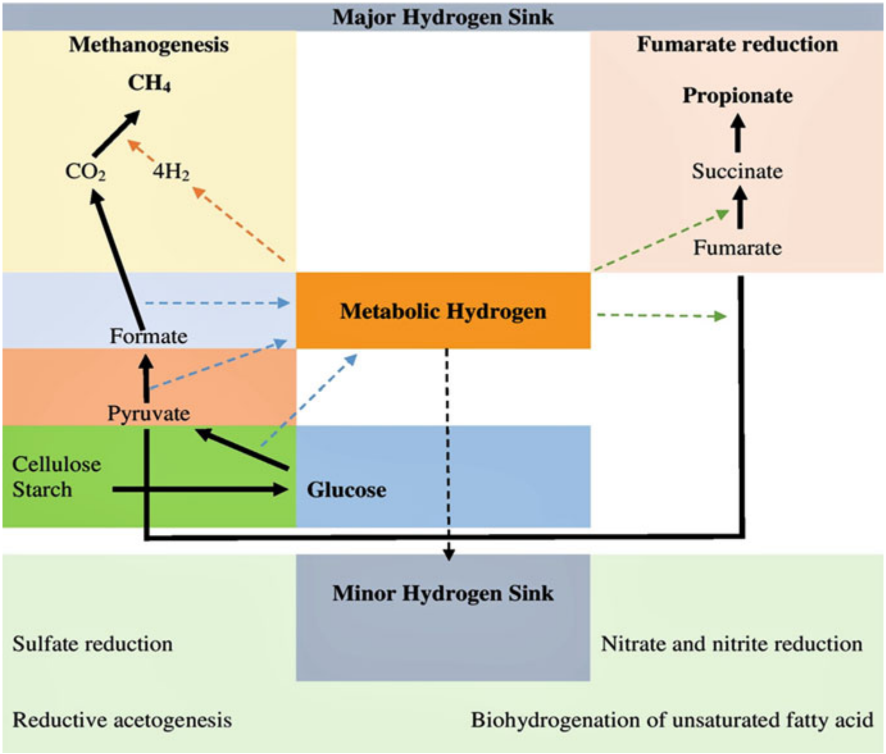


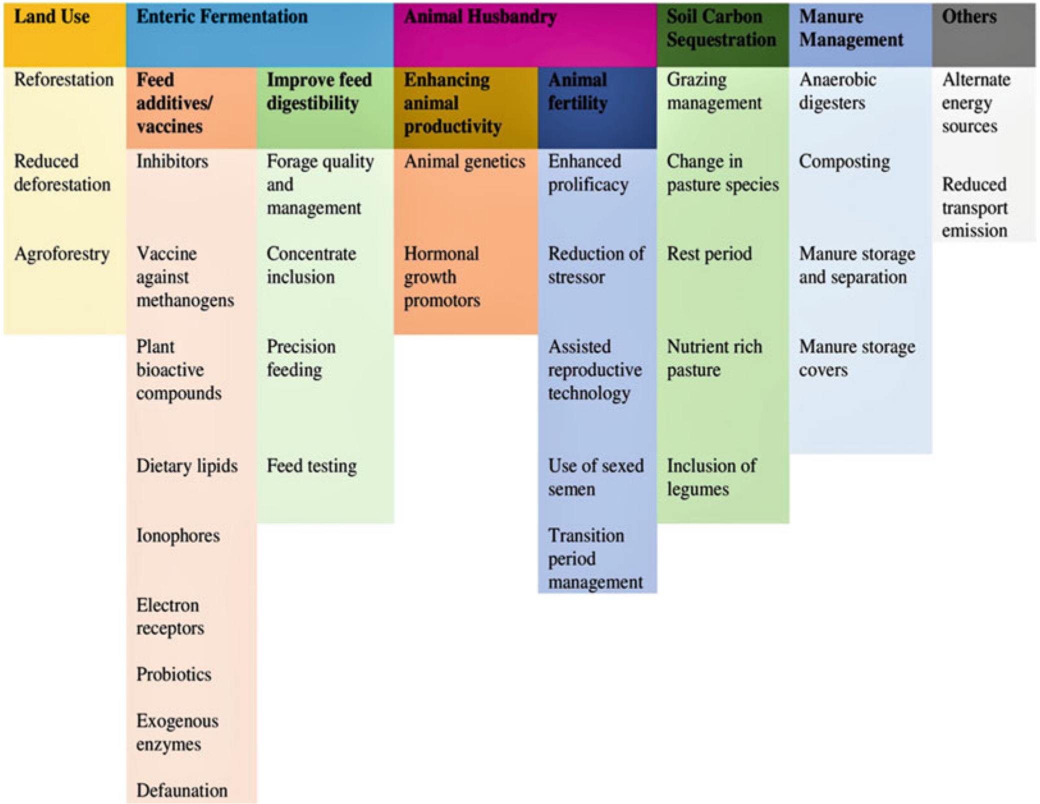
Fig. 3.2 Hydrogen-consuming pathways predictable in the rumen

chemicals and vaccines are directly active against methanogens or antibiotics and plant-originated metabolites indirectly affect methanogenesis.

3.5 Possible Strategies to Mitigate Greenhouse Gases from Livestock

The technical possibilities for mitigating greenhouse gas from the livestock sector have been the area of interest as reviewed recently by various scientific experts (Hristov et al. 2013b; Smithers 2015; Herrero et al. 2016; Rivera-Ferre et al. 2016).

They were either targeting reductions in enteric methane, reductions in nitrous oxide through manure management, sequestering carbon from pastures, or implementation of best animal husbandry and management practices that would have an effect on most greenhouse gas and land use practices, which also help sequester carbon (Fig. 3.3). Some of the better tested strategies for mitigation of enteric methane emission from livestock segments are described below (revised after Hristov et al. 2013b; Herrero et al. 2016; Smith et al. 2014).



Land Use	Enteric Fermentation		Animal Husbandry		Soil Carbon Sequestration	Manure Management	Others
Reforestation	**Feed additives/ vaccines**	**Improve feed digestibility**	**Enhancing animal productivity**	**Animal fertility**	Grazing management	Anaerobic digesters	Alternate energy sources
Reduced deforestation	Inhibitors	Forage quality and management	Animal genetics	Enhanced prolificacy	Change in pasture species	Composting	Reduced transport emission
Agroforestry	Vaccine against methanogens	Concentrate inclusion	Hormonal growth promotors	Reduction of stressor	Rest period	Manure storage and separation	
	Plant bioactive compounds	Precision feeding		Assisted reproductive technology	Nutrient rich pasture	Manure storage covers	
	Dietary lipids	Feed testing		Use of sexed semen	Inclusion of legumes		
	Ionophores			Transition period management			
	Electron receptors						
	Probiotics						
	Exogenous enzymes						
	Defaunation						

Fig. 3.3 Indicates enteric fermentation, soil carbon sequestration and soil management

3.5.1 Application of Halogens and Other Chemicals

According to Gribble (2004), halogens refer to those elements which hold negative electron affinity and pursue to associate with other compounds to reach stability through satisfaction of the valence shell in the rumen ecosystem. Bromoform and chloroform are halogens found to hinder directly with the methanogenesis pathway by serving as competitive inhibitors (or analogues), which prevents the final catalysis step (Goel et al. 2009). The mechanism which responding with reduced cyanocobalamin and preventing the cobamide-dependent methyl-transferase step of methanogenesis (Chalupa 1977); whereas, vitamin B_{12}-dependent methyltransferases also performs significant role in one carbon metabolism in acetogenic bacteria (Banerjee and Ragsdale 2003), thus halogenated compounds might have effect on reductive acetogenesis. According to Ungerfeld et al. (2004), some chemicals like 2-bromoethanesulphonate (BES), limazine, propionic acid and ethyl 2-butynoate inhibited methane synthesis from *Methanobrevibacter ruminantium*, *Methanosarcina mazei* and *Methanomicrobium mobile* in rumen, whereas compassion of the methanogens to individual chemical was species-dependent. Moreover, sensitive methanogens can be swapped by resistant methanogens by the use of chemicals over a certain period of time.

However, Denman et al. (2007) suggested that by offering bromochloromethane (BCM) to cattle, relative methane emission was reduced by 30% with enhancement of propionate and branched chain fatty acids as an alternate hydrogen sink, which also led to broadening of the methanogen numbers, and its application also suggests that alternative methanogens (*Methanomicrobium*, *Methanosarcina*, *Methanococcus* and unknown methanogens) are established following the suppression of chief methanogens like *Methanobacterium* by BCM. Therefore, alteration in methanogens diversity in response to an inhibitor should be considered as a probable vital mitigation strategy by chemical interventions. Villar et al. (2020) imply that the dietary use of nitrate in ruminant can also become an effective methane mitigation approach because nitrate competes with methanogens for hydrogen in the rumen, and this is a viable pathway of hydrogen utilisation by the methanogens in rumen due to larger changes in Gibbs energy than with methanogenesis.

Some developed countries are also working on some newer aspects as well to curtail the enteric methane emission. An investigation held in Japan involved two potential natural substances for diminishing rumen methanogenesis such as plant-derived liquid (PDL) and yeast-derived surfactant (YDS). PDL comprises anacardic acid (salicylic acid derivative with an alkyl group) which inhibits Gram-positive bacteria together with *Bacilli* and *Staphylococci* (Kubo et al. 1993). PDL is expected to typically deter Gram-positive rumen bacteria. It has been observed that both materials have influenced a dramatic reduction in methane emission in batch cultures (>95%) and in RUSITEC (>70%) without any contrary consequence on feed digestibility or total volatile fatty acid production. Although yeast-derived surfactant interrupts bacterial cell walls liable on the structure of the bacterial surface selectively and Gram-negative bacteria have an outer membrane which reduces bacterial cell impairment from such surfactant, consequently it might also selectively prevent Gram-positive rumen bacteria. Moreover, YDS and PDL revealed similar antibacterial effect when inspected against descriptive rumen bacterial species. Some of the propionate and succinate producer rumen microbes like *Selenomonas ruminantium*, *Megasphaera elsdenii* and *Succinivibrio dextrinosolvens* were observed to be tolerant to these two substances, whereas hydrogen and formate producers microbes such as *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Butyrivibrio fibrisolvens* and *Eubacterium ruminantium* were sensitive (Kubo et al. 1993). Hence, application of both materials are supposed to alter rumen fermentation toward more propionate and less methane production through selective antibacterial activities, but further assessment of these substances as an additive needs to be explored by performing animal trials.

3.5.2 Probiotics Supplement

On livestock farms, using live microbial feed to stimulate the rumen microbiota for better digestion and nutrient utilisation is a routine trend. In vitro and in vivo trials, however, providing live microbial feed to reduce methane production have not proven consistent (Bayat et al. 2015). Addition of *Saccharomyces cerevisiae* reduced methane emission by 50% due to a decline in protozoal population (Frumholtz et al. 1989), but addition of *Aspergillus oryzae* reduced methane release by 10% in vitro, which did not last long (Mutsvangwa et al. 1992). According to Eun et al. (2003), brewer's yeast culture enhanced the activity of bacteria that convert hydrogen to acetate and diminished methane emission by 25% in a continuous culture system, although more in vivo research is needed. The precise mode of action is still unclear, because livestock farmers are unfamiliar with the benefits of probiotics; it is critical to discover the dietary and managerial conditions under which probiotics can provide consistent production benefits as well as the added benefit of lowering methane emissions.

3.5.3 Hydrogen Receptors

According to Joblin (1999), the production of acetic acid by acetogens, and in the guts of termites and rodents, acetogens convert surplus hydrogen to acetic acid, which can then be used by the host, is one of the aids that includes some ability in the long run of diverting electrons from methanogens. Ruminants, on the other hand, contain less acetogens, which cannot effectively compete with methanogens for hydrogen ions because they have a lower affinity for hydrogen than methanogens (Nollet et al. 1998). As a result, methanogens must be inhibited in order for hydrogen pressure to rise for acetogenesis to become a viable alternative hydrogen sink in the rumen. However, exogenous inoculations of acetogens into the rumen, on the other hand, could be beneficial for competing against methanogens.

3.5.4 Application of Ionophores

Since the mid-1970s, ionophores like monensin have been widely used as ruminant feed additives all over the world. Monensin alters rumen microbial communities to improve gut efficiency by limiting methanogen substrates that Gram-positive bacteria and ciliate protozoa normally offer (Hook et al. 2010). It has beneficial effects on rumen fermentation, including methane reduction, propionate augmentation, and ammonia reduction, as well as preventing coccidiosis, bloat and lactic acidosis, which is attributed to monensin's specific antimicrobial activity on rumen bacteria. According to Odongo et al. (2007), long-term administration of monensin to dairy calves reduces methane emissions by 7% while having no negative impact on milk yield. Furthermore, the administration of ionophores in ruminants reduces protozoal population, resulting in a reduction in methane since protozoa accommodate methanogens on their cell surface and within the cell (Tokura et al. 1997). Although, the most common fibrolytic bacteria (*Fibrobacter succinogenes*) appears to be resistant to monensin, as evidenced by its prevalence in the rumen as assessed by DNA probing (Stahl et al. 1988).

3.5.5 Dietary Lipids

Dietary lipids have a variety of effects on the rumen environment, including toxic effects on methanogens and protozoa, biohydrogenation of unsaturated fatty acids and a shift to propionate synthesis, which reduces enteric methane production (Beauchemin et al. 2008, 2009). However, the efficiency of lipids in reducing methane generation is dependent on the source, type and dosage (Beauchemin et al. 2008; Eugene et al. 2008). Rumen methanogenesis is known to be inhibited by plant oils rich in medium-chain fatty acids (Dohme et al. 2000). Medium-chain fatty acids including lauric, myristic, capric and caprylic acids reduced methane emission by 3%, 37% and 45%, respectively.

Bayat et al. (2015) discovered that including polyunsaturated fatty acids sources such as camelina oil (60 g/kg dry matter) in the diet reduced enteric methane production by 29.5% while having no effect on milk yield or components. Furthermore, supplementing cottonseed oil with meals has been demonstrated to reduce enteric methane output by about 42% (Nogueira et al. 2020). Furthermore, most oils and fatty acids that reduce methanogenesis also reduce rumen protozoal numbers, which have a symbiotic relationship with methanogens, so the decrease in rumen protozoal numbers is partly responsible for the decreased methane production induced by oils and fatty acids application in ruminants.

3.5.6 Plant Bioactive Compounds

Essential oils, saponin, tannins, flavonoids and other plant bioactive components have been investigated for their potential capacity to inhibit rumen methanogenesis. Saponins also have a detergent effect, disrupting microbial cell membranes by forming a complex with membrane sterols. Protozoa in the rumen are extremely sensitive to saponins, resulting in a decrease in protozoal population and, as a result, a decrease in the number of methanogens in the rumen (Guo et al. 2008). However, Poornachandra et al. (2019) observed that supplementing cross-bred cattle with soapnut, a saponin-containing plant, did not lead to a considerable reduction in methane output.

Tannins are polyphenolic compounds that have been shown in vivo and in vitro to reduce methane production. According to Carulla et al. (2005), the inhibition of methanogens by condensed tannin is due to reduced fibre degradation, which limits the hydrogen derived from acetate synthesis. The reduced fibre degradation could be due to a decreased number of cellulolytic bacteria; the formation of tannin-cellulose complexes, which impaired bacterial adhesion to substrate; and rumen microbes' fibrolytic activity. According to Patra et al. (2017), condensed tannins containing forage reduced methane output by goat; nevertheless, the effect of condensed tannins on methane emission is due to variations in archaeal activity, which could possibly involve changes in protozoal activity. As a result, they proposed that somewhat moderate dietary levels of condensed tannins may be given to animals to reduce methane emissions without causing any significant physiological impairment.

Flavonoids have antimicrobial properties, but not known to be a substantial methane-mitigating agent (Patra and Saxena 2010). Flavonoids have been studied in vitro to obtain a better knowledge of their antibacterial characteristics and relationship to methanogenesis, although animal studies are rare (Oskoueian et al. 2013; Kim et al. 2015). Meanwhile, Kim et al. (2015) showed that flavonoids lower methane production by 39–48% in vitro, although these findings have yet to be replicated in animal trials. According to Stoldt et al. (2016), feeding rutin trihydrate to dairy cattle at a dose of 100 mg/kg live weight increased plasma glucose, b-hydroxybutyrate and albumin levels, but did not conquer methane production.

Essential oils are naturally occurring substances isolated from plants possessing volatile properties that are commonly used in cosmetics, but to a lesser extent in animal and human pharmaceutical sectors (Benchaar et al. 2008). Antibacterial activity is mainly attributed to the key components of essential oils, which are classified as terpenoids or phenylpropanoids. Their antibacterial spectra are quite broad, and their mechanism of action involves antibacterial compound interaction with the bacterial cell membrane, which destabilises the membrane. Several plants, including cinnamon, lemongrass, ginger, garlic, fennel, clove, onion, eucalyptus, thyme, citrus, oregano, mint, rosemary and coriander, have been screened in vitro, but only a few plant sources have been investigated on animals, according to Nanon et al. (2015). Due to the non-specific characteristics of essential oils, their presence in the diet may have an adverse effect on the microbial habitat, resulting in a decrease in animal feed efficiency. Hristov et al. (2013a) observed that supplementing dairy calves with *Origanum vulgare* leaves lowered methane generation by up to 40% while having no negative consequences on feed efficiency, rumen pH or volatile fatty acid concentrations.

Although ruminal protozoa provide a habitat for methanogens that live on and within them, several essential oil constituents favour methane reduction via selective inhibition of protozoal populations, hence lowering methane generation. Garlic contains organosulfur compounds, predominantly diallyl disulfide, which limit methanogen enzymic activity while somehow reducing protozoal count (Soliva et al. 2011). Furthermore, feeding varying amounts of eugenol, cinnamaldehyde and cinnamon oil to dairy cattle did not significantly reduce methane generation (Benchaar 2016). According to Hart et al. (2019), commercially available essential oil blends with antimicrobial properties (Agolin Ruminant, Switzerland), which include eugenol, geranyl acetate and coriander extract have shown a nearly 20% reduction in methane intensity in dairy calves. As a result, it appears that selective essential oil molecules that selectively reduce methane can be employed at levels that do not reduce feed utilisation; nevertheless, more research is needed in animal experiments to evaluate these compounds.

3.5.7 Archaeal Viruses

It is well-known that bacteriophages are obligatory and highly host-specific pathogens that may infect and lyse bacteria as well as methanogens. Although bacteriophages are well-known in the rumen (Mackie and White 1990), evidence on archaeal viruses is still scarce. There are still no viruses recognized from rumen archaea, and only a minority from methanogens in general (Klieve and Hegarty 1999). As a result, they suggest that assessing the potential of archaeal viruses as a biological control agent is difficult without a significant increase in understanding about methanogen genetic diversity and viral susceptibility, as well as the host range of archaeal viruses.

3.5.8 Vaccination

To minimise enteric methane emissions, some developed countries are performing vaccine trials in vitro and in vivo under controlled and field conditions against methanogens. According to Wright et al. (2004), a vaccination method resulted in a significant 7.7% reduction in rumen methane production, with less than 20% of the methanogens targeted by the vaccine developed using three *Methanobrevibacter* strains of archaea. According to Williams et al. (2009), a wider-range vaccine was developed to bring a broader scope of methane reduction, and this vaccine (targeting >52% of different species/strains of methanogens) was administered to 32 sheep, where specific IgG titres in plasma, saliva and rumen fluid had no effect on methane output or the rumen methanogen population. However, the growth of alternative methanogens following immunisation could be a contributory cause in this failure, necessitating a much larger approach as well as a comprehensive understanding of the rumen methanogen population for successful immunization to combat global challenges.

3.5.9 Escalation of Animal Diets

It has been established that offering higher-quality feed to animals reduces methane and other greenhouse gas production per unit of animal product (Gerber et al. 2013). This can be addressed through better supplementation approaches or better land use management strategies, such as grazing rotation, fertilizer use, soil pH change, fodder banks, upgraded pasture species, mixed cropping systems, and the use of superior crop by-products. As a result, the amount of methane in overall emissions falls, while the share of emissions associated with feed production rises. The changes in land utilization, carbon dioxide emissions increased, whereas methane emissions per unit of output declined (Gill et al. 2010). However, the largest greenhouse gas efficiency gaps are seen in livestock systems with the poor diet quality and the highest peripheral lifting of changing animal diets with simple feeding methods, which had both biological and economic impacts in these systems (FAO 2013; Herrero et al. 2013).

3.5.10 Reducing Livestock Population Following Improved Management Practices

One of the primary problems is the increase in animal population, which directly contributes to greenhouse gas emissions (EPA 2012; Thornton and Herrero 2010). With improved grazing management, many low-producing animals are being replaced in many developing countries by fewer animals with higher production potential. Such approaches have the potential to lower total emissions while also boosting the availability of livestock products for consumers. Furthermore, improvements in animal health can dramatically reduce emission intensity by increasing production and fertility per animal and decreasing mortality (ADAS 2015).

3.5.11 Genetic Selection

According to Robertson and Waghorn (2002), Dutch cross Holstein cows produced 8–11% less enteric methane than New Zealand Friesian cows for about 150 days post calving fed either on grazing or offering total mixed ration, indicating that enteric methane production could vary with breeds and genetic merits of animals. As a result, selecting animals with a faster rumen feed passage rate would reduce methane emissions per unit of feed ingested, as well as propionate and microbial output, resulting in significant production improvements. Identifying animals with a high net feed efficiency (NFE) could, however, be a way to reduce daily methane emissions without decreasing livestock numbers.

3.5.12 Manure Management

Poor manure management in developing world wastes a considerable amount of nutrients. However, reducing greenhouse gas emissions in the livestock sector is a challenging task that necessitates systems thinking and a knowledge of key driving forces in various livestock systems. The nitrogen emission through excreta can be reduced by providing balanced feeds to livestock, which is easily lost as ammonia and enters the nitrogen cascade (Bouwman et al. 2013). Whereas the nitrogen can be captured efficiently using bedding material in intensive farming practices, handling manure as slurry in tanks or anaerobic lagoons can reduce direct nitrous oxide emissions during storage but increase methane and ammonia loss, as well as increase the risk of emissions during land spreading (Velthof and Mosquera 2011). Therefore, optimizing manure distribution to promote nitrogen and phosphate replacement value can help reduce ammonia losses (Bourdin et al. 2014). Ammonia and nitrous oxide emissions can be controlled in a large-scale farming system by using spatially shifting animal pens.

3.6 Conclusion

The similarities and variations between methanogens and metabolic pathways in animal guts and enteric methane became a worldwide concern. Methane synthesis in the rumen can be reduced by gradually shifting fermentation toward propionate formation, with no negative impact on ruminant animal production performance. The use of a diverse range of feed additives, including plant bioactive substances, offers a great chance to improve the long-term production of animal-origin foods by considerably lowering enteric methane emissions. Furthermore, any effective strategy, whether chemical or biotechnological, must incorporate the provision of alternate electron acceptors inside the rumen in order to change the fermentation pattern. However, it should be remembered that pursuing a significant reduction in enteric methane emissions should be a moral endeavour, in which the value of ruminants in terms of converting fibrous feeds inappropriate for human consumption into high-quality protein sources such as meat and milk must be weighed against the contribution of the arising methane emissions to global warming.

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Chapter 4
Rumen Microbiology and Microbial Degradation of Feedstuffs

Vinod Kumar Paswan, Kaushalendra Kumar, and Abdelrazeq M. Shehata

Abstract The mixed and complex microbial ecosystem of the rumen comprises numerous interactions between its anaerobic inhabitants, viz., bacteria, protozoa, fungi, archaea, and also the bacteriophage. The rumen is a dynamic system open to the external environment, but the ruminal microbial niche is maintained at a constant milieu well suited for these diverse anaerobic rumen microbial population to grow and multiply. Temperature, pH, buffering capacity, osmotic pressure, and redox potential are the primary factors affecting the growth and activity of these microbes. Efficient microbial fermentation inside the rumen depends on the actions of diverse enzymes secreted by these microorganisms on the complex feed compounds. Cellulase, hemicellulose, esterase, and pectinase from the fiber digester microbes (bacteria, protozoa, and fungi) ferment complex structural carbohydrates. In addition, these microorganisms ferment starch and soluble sugars, protein, and non-protein nitrogen substances. Certain ruminal microbes are also capable of detoxifying potentially toxic substances in feed such as phytotoxins, anti-nutritional factors, and mycotoxins. Ruminal archaea or methanogens create an environment conducive for other ruminal microbial population by capturing hydrogen and converting them to methane. However, methane is a potent greenhouse gas which is a major global environmental concern, and efforts are going on to find effective mitigation strategies. Anaerobic rumen fungi perform important function in fiber degradation by splitting feed particles through mycelial development, by firmly attaching to these lignified feed particles through their rhizoid, and by exposing them for the action of fibrolytic enzymes of the ruminal microbes. Ruminal bacteriophages are the least explored among all the rumen microbes, but they provide

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_4

enormous scope for their wider use such as controlling and eliminating certain ruminal microbes and their applications in treatment through the novel branch of phage therapy.

Keywords Rumen microbes · Fiber degradation · Ruminal fermentation · Methanogens · Ruminant digestion

4.1 Introduction

Rumen is the first and the largest of the four chambers of ruminant foregut. Pre-gastric fermentation of fibrous feedstuffs ingested by animals takes place in this chamber by the complex microbial interactions between a range of anaerobic microorganisms comprising bacteria, protozoa, fungi, and primitive archaea and also the bacteriophage (Forsberg and Cheng 1992). This mixed and complex microbial ecosystem not only facilitates growth and multiplication of the microbes involved but also facilitates nutrient derivation and energy for host animals. The primary source of energy and other nutrients on a forage-based diets of ruminant animals consists mainly of the structural polysaccharides of the plant cell walls such as cellulose, hemicellulose, and pectin, in addition to other non-structural polysaccharide such as starch. The structural complexity and insolubility of recalcitrant cell wall components of the forage-based feedstuff limit their degradation and ruminal fermentation (Nagaraja et al. 1997). However, the degradation and metabolism of these structural carbohydrates are achieved in the complex ruminal microbial ecosystems through the synchronous activities of the diverse microbial enzymes secreted by the ruminal microbes interacting mostly in a synergistic relationship (Burns 2008). Microbial fermentation of ingested structural carbohydrates is vital for digestion and metabolism of feedstuffs by the host animal yielding volatile fatty acids such as acetate, butyrate, propionate, formic acid, methane, H_2, and CO_2 (Krause et al. 2003). These short-chain fatty acids mainly acetate, propionate, and butyrate are rapidly absorbed by the rumen epithelium and make up to 80% of the energy requirements of the ruminant host (Bergman 1990; Gäbel and Sehested 1997).

4.2 Ruminal Environment and Microbial Niche

The ruminal microbial niche is almost a constant environment well suited for large and diverse anaerobic ruminal microbial population to grow and multiply, making it a highly efficient continuous culture system for these microbes. Food and water supply, pH and buffering capacity, temperature and osmotic pressure, type and strength of microbial culture, outflow of end products of fermentation, undigested residues and microorganisms, all are fairly constant in the ruminal milieu (Wang and McAllister 2002). The digestive tract of ruminant animal constitutes quite larger proportion of the total body weight (about 40%), and volume of the reticulo-rumen

Table 4.1 Summary of physical and chemical characteristics of the rumen ecosystem

Physical criteria	Range characteristics
pH	5.5–6.9 (mean 6.4)
Redox potential	−350 to −400 mV
Temperature	38–41 °C
Osmolality	250–350 milliosmole/kg^{-1}
Dry matter	10–18%
Chemical criteria	Range characteristics
Gas phase (%)	CO_2, 65; CH_4 27; N_2 7; O_2 0.6; H_2 0.2
Volatile fatty acids (mmol L^{-1})	Acetate 60–90; propionate 15–30; butyrate 10–25; branched chain and higher 2–5
Nonvolatile acids (mmol L^{-1})	Lactate <10
Amino acids and oligopeptides	<1 mmol L^{-1} present 2–3 h post feeding
Ammonia	2–12 mmol L^{-1}
Soluble carbohydrates	<1 mmol L^{-1} present 2–3 h post feeding
Insoluble polysaccharides	
Dietary (cellulose, hemicelluloses, pectin)	Always present
Endogenous (mucopolysaccharides)	Always present
Lignin	Always present
Minerals	High Na; generally good supply
Trace elements/vitamins	Always present; good supply of B vitamins
Growth factors	Good supply; branched-chain fatty acids, long-chain fatty acids, purines, pyrimidines, other unknown

Source: adapted from Mackie et al. (1999)
Note: mmol L = millimole per liter; mV = millivolts

makes up about 85% of the total capacity of the digestive tract. Temperature, pH, buffering capacity, osmotic pressure, and redox potential are the primary factors affecting the growth and activity of ruminal microbial populations (Russell and Rychlik 2001) (Table 4.1). The rumen temperature is typically regulated in the range of 38–41 °C and has an oxidation potential of −350 mV (Choudhury et al. 2015). Strict anaerobic conditions (−150 to −350 mv) required for the efficient microbial fermentation in the rumen are preserved by the facultative anaerobic bacteria of the ruminal wall which quickly utilizes the traces of oxygen entering into the rumen through feed or water or through diffusion across the ruminal wall (Clarke 1977). In spite of the large volume of liquid consumed through feed, drinking water, or saliva, the dry matter content of the rumen is maintained at a fairly constant range of 10–18%. This not only provides a liquid environment but also ensures a sustained optimal supply of substrates necessary for efficient and continuous microbial fermentation. Further, frequent and regular rumination (i.e., regurgitation, re-mastication, and re-swallowing of coarse foregut digesta by the ruminant animals) enhances the process of fermentation by reduction in particle size of the coarse fibers and increases surface area of feed particles for microbial enzymatic exposure and action. Although the physiological pH is among the most variable physicochemical factors of the ruminal environment (Russell and Strobel 1989), the rumen content has a high buffering capacity, and pH of the ruminal content is maintained within a fairly constant range of 5.5–7.0. (Dehority 2003; Krause and Oetzel 2006). The pH and buffering capacity in the reticulo-ruminal environment are determined by the amount of saliva and its bicarbonate and phosphate content, quantity and the nature of the feedstuffs ingested by the host ruminant, production and absorption of the short-chain fatty acids, and neutralization and absorption of the bicarbonates and phosphates in the rumen (Krause and Oetzel 2006). Exo-enzymes secreted by the ruminal microorganisms which degrade the feedstuffs and perform microbial fermentation are sensitive to the changes of ruminal pH. Similarly, the normal neutral intracellular pH of rumen microorganism is affected greatly by the H^+ ion imbalance caused by the drop in pH due to high VFA and LA formation on high starch-based diets (Russell and Wilson 1996) which inhibits the microbial function and growth.

The osmolality of the ruminal fluid is about 250 mOsm/kg, and it depends on the concentrations of ions and molecules which generate gaseous tension and influences pH due to VFAs formation (Lodemann and Martens 2006). Further, the osmotic pressure inside the rumen is affected by the nature of diets and other environmental factors inside the rumen which influence the microbial fermentation. Immediately after feed intake, the osmotic pressure increased from 350 to 400 mOsm and then decreases gradually over the next 8–10 h (Lodemann and Martens 2006).

The microbial fermentation inside the rumen depends on the actions of enzymes secreted by the anaerobic microorganisms interacting in synergistic, synchronized, and systematic way on the complex food compounds. Actions of cellulase and hemicellulase degrade complex structural polysaccharides of plant cell wall to produce VFAs to be further utilized as source of energy by the host animals (Burns 2008; Russell and Mantovani 2002). Further, several microbial proteases and deaminases act on low-quality protein of the roughage-based feedstuffs and non-protein nitrogenous substances to produce ammonia and their further uptake and utilization for de novo synthesis of microbial proteins. The microbial proteins are digested further by the host animals inside the abomasum—the true stomach—and small intestine from which all of the essential amino acids required by the host animals are derived (Cole et al. 1982). Additionally, certain ruminal microbes are also capable of detoxifying potentially toxic substances in feed such as phytotoxins, anti-nutritional factors, and mycotoxins to harmless intermediates that have no adverse effect on the animals' health. Nevertheless, ruminal fermentation processes cannot be totally efficient as it generates some by-products such as methane gas and excess ammonia. Thus, in a mutualistic and symbiotic relationship where microorganisms provide all the essential nutrients to the host animals, the latter provide all the substrates and the suitable environment for establishment of the microbial niche in which proper growth and multiplication of microorganisms and efficient microbial fermentation take place (Russell and Rychlik 2001).

4.3 Rumen Microorganisms and Degradation of Feedstuffs

Much of our understanding about the anaerobic microbial ecology of the rumen is credited to Hungate—the father of anaerobic microbiology and the father of rumen microbiology, who invented the roll tube technique for isolation and culture of anaerobic rumen microorganisms during the 1950s (Hungate 1947, 1950; Hungate 1957). This technique provided much of the insight about the anaerobic ruminal microbes and especially about rumen bacteria. The ruminal microflora consists of a wide range of obligate anaerobic microorganisms occurring in a symbiotic and mutualistic relationship with host ruminant to perform microbial fermentation. After separating the ruminal methanogens from bacteria (Woese et al. 1990), all these ruminal microorganisms can be classified into the following five groups: bacteria, ciliate protozoa, anaerobic fungi, methanogens (archaea), and bacteriophages. These microorganisms are present in the rumen in different concentrations and perform various functions (Table 4.2), all of which contribute toward efficient anaerobic fermentation, making the rumen an ideal fermentation vat.

The rumen is a dynamic system, and the inhabiting microorganisms continuously adapt themselves to the form and physical structure of the ingested feedstuffs, quantity and frequency of consumption, changes in the ingredients composition of the diet, and its chemical and nutritional composition. Just after ingestion of feedstuffs, microorganisms quickly associate themselves with different components of the rumen content and ingested feed particles (Craig et al. 1987; Bonhomme 1990). The slow-working fiber-digester microbes attach themselves to the fibrous materials and digestible dry matter on the fiber mat of the dorsal rumen contents, and the fast-working microbes which ferment sugars and starches associate themselves in the rumen fluid in ventral contents. Bacteria in the ventral ruminal fluid has the mean

Table 4.2 Anaerobic microbes of the rumen, their concentrations, proportions, and important functions

Microbes	Concentration	% of cell number[a]	% of microbial weight	Functions
Bacteria	10^{10}–10^{11} g^{-1} (> 200 species)	~98%	50–60%	Ferment fiber, starch, sugars, protein, and other substances
Archaea	10^{7}–10^{8} g^{-1} (25 genera)	1%	1–3%	Produce methane gas
Ciliate protozoa	10^{4}–10^{6} g^{-1} (25 genera)	1%	40–50%	Ferment starch, fiber, and feed upon bacteria
Fungi	10^{3}–10^{5} g^{-1} (5 genera)	<1%	5–8%	Break down fiber, facilitates action of microbial enzymes on fibers
Bacteriophage	10^{7}–10^{9} g^{-1}	–	< 0.1%	Scavenge rumen bacteria and archaea

Source: Castillo-González et al. (2014), Agarwal et al. (2015)

[a] Excluding bacteriophage concentration

generation time of 7 h, while bacteria present on dorsal ruminal mat have comparatively slow passage rate and longer generation time (Bryant 1970). As an integral part of the rumen ecosystem, microorganisms associated with the ruminal fluid survive on soluble feed components and colonize and initiate digestion of newly ingested feed particles. Further, loosely associated bacteria and firmly attached microbes which account for 70–80% of the microbial population in the rumen are responsible for 70–80% of the amylase, protease, and endoglucanase activity (Minato et al. 1966; Brock et al. 1982) and comparatively higher hemicellulase and cellulase activities in the ruminal fibrous mat (Williams and Strachan 1984). Fungal zoospores chemotactically attach rapidly to plant-based fibrous materials of the dorsal mat and break these fibrous materials through their penetrating branching mycelia which grow afterward. This increases the area of exposed substrate combined by the joint effect of slow passage rate and prolong residency of the insoluble fibrous feed particles, which increases the reaction time between substrates and fungal and other enzymes from some other fiber fermenter bacteria and protozoa (Orpin 1975; Williams and Orpin 1987).

4.4 Rumen Bacteria

4.4.1 Fiber and Starch Degrading Bacteria

Efficient digestion of complex fibrous substrates in the rumen requires the combined and coordinated actions of all the ruminal microorganism and their enzymes. However, the collective activities of bacteria, fungi, and protozoa in fiber degradation are particularly important as 80% of degradation is performed by bacteria and 20% by protozoa (Dijkstra and Tamminga 1995). *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* are the three most important bacteria which perform fiber degradation in the rumen (Cheng et al. 1991; Forsberg and Cheng 1992). Efficient fiber degradation depends on the optimum ruminal environmental condition, nature of the fodder, its stage of maturity, rate of passage of digesta, microbial communities involved, their attachment with the fibrous feed, and the nature and extent of cellulolytic, hemicellulolytic, and pectinolytic enzymes secreted (Fondevila and Dehority 1996; Mitsumori and Minato 1997). However, fiber degradation is also affected by other diverse microorganisms which utilizes starch, sugars, lipids, proteins, phytates, and other anti-nutritional compounds present in feed and fodders (Table 4.3). Also important are bacteria which thrive on and utilize fermentation end products of other microorganisms such as lactate-degrading bacteria, acetogens, acid utilizers, etc. (Castillo-González et al. 2014). The action of amylolytic and saccharolytic bacteria in the rumen is also important as high milk yielding cattle are fed on concentrates containing large proportions of grains. Starch and sugars are the readily fermentable source of energy for ruminants, and the major bacteria which thrive on them are *Streptococcus bovis, Bacteroides ruminicola*, *Ruminobacter amylophilus*, *Selenomonas ruminantium*, *and Succinomonas*

Table 4.3 Different types of anaerobic rumen bacteria, the enzymes involved, and the end products of fermentation

Type of rumen bacteria	Important bacterial species	Enzymes involved	Fermentative products
Fiber degraders			
Cellulolytic	*Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, *R. albus*, *Clostridium cellobioparum*, *C. longisporum*, *C. lochheadii*, *Eubacterium cellulosolvens*	Endo-β-1,4-glucanase Exo-β-1,4-glucanase β-1,4-Glucosidase Cellulodextrinase O-Acetyl xylan esterase Ferulic acid esterase p-Coumaric acid esterase	Acetate, formate, lactate, butyrate, succinate, H_2, CO_2
Hemicellulolytic	*Prevotella ruminicola*, *Eubacterium xylanophilum*, *Eubacterium uniformis*	Xylocellulase Endo-β-1,4-xylanase β-1,4-Xylosidase α-L-Arabinofuranosidase α-Glucuronidase O-Acetyl xylan esterase Ferulic acid esterase p-Coumaric acid esterase	Acetate, formate, lactate, butyrate, succinate, H_2, CO_2
Pectinolytic	*Treponema saccharophilum*, *Lachnospira multiparus*	Pectin lyase Polygalacturonase Pectin methylesterase	Low-methoxyl pectin, polygalacturonic acid, disaccharides
Soluble carbohydrate (starch and sugar) fermenters	*Streptococcus bovis*, *Ruminobacter amylophilus*, *Prevotella ruminicola*, *Bacteriodes ruminicola*, *Succinomonas amylolitica*, *Succinivibrio dextrinosolvens*, *Selenomonas ruminantium*, *Lactobacillus acidophilus*, *L. casei*, *L. fermentum*, *L. plantarum*, *L. brevis*, *L. helveticus*, *Bifidobacterium globosum*, *B. longum*, *B. thermophilum*, *B. ruminale*, *B. ruminantium*	α-Amylase, maltase, invertase, sucrase, etc.	Formate, acetate, succinate, lactate, propionate, alcohols, H_2, CO_2
Proteolytic	*Prevotella ruminicola*, *Ruminobacter amylophilus*, *Clostridium bifermentans*	Proteases, peptidases, deaminases	Ammonia, amino acids, VFAs
Lipolytic	*Anaerovibrio lipolytica*	Lipases, biohydrogenases	Acetate, propionate, acetate

(continued)

Table 4.3 (continued)

Type of rumen bacteria	Important bacterial species	Enzymes involved	Fermentative products
Acid utilizers	*Megasphaera elsdeni*, *Wolinella succinogenes*, *Veillonella gazogene*, *Micrococcus lactolytica*, *Oxalobacter formigenes*, *Desulfovibrio desulfuricans*, *Desulfotomaculum ruminis*, *Succiniclasticum ruminis*	Lipases	Acetate, propionate, butyrate, succinate, Valerate, H_2, CO_2
Tanninolytic	*Streptococcus gallolyticus (Streptococcus caprinus)*, *Selenomonas ruminantium*, *Lonepinella koalarum*	Tannin acyl hydrolase (tannase)	Gallic acid, pyrogallol

Sources: Wang and McAllister (2002), Choudhury et al. (2015), Agarwal et al. (2015), Bhat et al. (1998)

amylolytica. These microorganisms ferment starch and sugars into VFAs such as acetate, propionate, butyrate, formate, and succinate. However, when large amounts of starch and sugars are introduced suddenly through a high grain-based diet, the ruminal pH drops below 5.5 and favors explosive growth of *Streptococcus bovis* which yields lactic acid as end product as against VFAs and alcohol on normal fermentation. This causes a further sudden drop of pH in the rumen and a potential lethal metabolic disorder of rumen called ruminal acidosis or lactic acidosis (Gressley et al. 2011). The condition may be avoided by gradual introduction of starch-based diet to facilitate growth of other amylolytic and saccharolytic bacteria and also lactic acid-degrading bacteria like *Megasphaera elsdenii*.

4.4.2 Proteolytic and Lipolytic Bacteria

There are bacteria which hydrolyze protein and non-protein nitrogenous substances of feedstuffs. About 60% of the proteins are degraded by ruminal proteolytic species *Bacteroides amylophilus, Bacteroides ruminicola, Butyrivibrio fibrisolvens*, *Streptococcus bovis*, and *Prevotella albensis* (Cotta and Hespell 1986; Sales-Duval et al. 2002). The degradation of proteins and NPN substances such as polypeptides, oligopeptides, amino acids, and urea causes production of ammonia, VFAs, and H_2S. Microorganisms utilize these and cause de novo synthesis of microbial protein. About 60% of protein needed by the host ruminant comes from the subsequent digestion of ruminal microorganisms in omasum and small intestine. Lipolytic microorganisms such as *Anaerovibrio lipolytica* causes lipolysis, and *Butyrivibrio fibrisolvens* causes saturation of unsaturated fatty acids by hydrogenation. Acidic and highly reductive environment of rumen, with surplus of H_2 coupled with the actions of microbial lipases and hydrogenase from these microorganism, favors saturation of polyunsaturated fatty acids (Maia et al. 2010).

4.4.3 Other Important Ruminal Bacteria

Lactate-degrading bacteria have important role in utilizing lactic acid, which is an intermediate product of fermentation in ruminants fed on high grain-based diets (Agarwal et al. 2015). As mentioned earlier, *Megasphaera elsdenii* is the main lactate degrader and is helpful in preventing ruminal acidosis when high grain diets are introduced gradually over an extended duration for adaptation on such diets. Further, there are important bacterial species, which causes degradation of pectin by pectinolytic enzymes, viz., pectin lyases, polygalacturonase, and pectin methylesterase (Dušková and Marounek 2001; Gordon and Phillips 1992). The important pectin-degrading bacteria are *Butyrivibrio fibrisolvens*, *Bacteroides ruminicola*, *Prevotella ruminicola*, and *Lachnospira multiparus*. There are some important bacteria which scavenge reducing potential through alternate hydrogen sink. These include acetogenic bacteria such as *Acetitomaculum ruminis* and *Eubacterium limosum* which reduce CO_2 to form acetate, a process which is called as reductive acetogenesis. Other alternate sinks are sulfate-reducing bacteria and bacteria which reduce fumarate to succinate (Asanuma and Hino 2000). Tannin-degrading bacteria such as *Selenomonas ruminantium* (Odenyo and Osuji 1998) and *Streptococcus gallolyticus* (Singh et al. 2011) with significant tannase activity and utilizing tannin as energy source have been identified. Similarly, mimosine-degrading bacteria *Synergistes jonesii*, isolated from rumen of Hawaiian goats, hydrolyze mimosine—a toxic phytochemical present in *Leucaena leucocephala* leaves—into a nontoxic compound, viz., 2,3-DHP (Allison et al. 1992).

4.5 Archaea

Archaea or methanogens represent about 3–4% of the total rumen microbial population (Sharp et al. 1998; Ziemer et al. 2000). Most of the methanogens remain free floating in the rumen fluid or stick to the feed particles, whereas some methanogens are ecto- or endosymbionts to other rumen microbes particularly bacteria and protozoa (Belanche et al. 2014; Valle et al. 2015). Although ruminal archaea constitute a minor proportion of the ruminal microbial population, but by capturing H_2 and converting them to CH_4, they create a ruminal environment conducive for efficient fermentation of the key nutrients by the other ruminal microbial population. The main fermentation end products of fiber, starch, sugars, and proteins from ruminant diets are VFAs, NH_3, CO_2, and H_2. While, VFAs and NH_3 are utilized as energy source and synthesis of microbial proteins, H_2 and CO_2 are taken up by these methanogens for conversion into methane (van Zijderveld et al. 2011). Although in ruminants methane production is the main sink of H^+ (Moss et al. 2000), it is a wasteful process in which 2–12% of gross energy is wasted through methane emission (Johnson and Johnson 1995). In livestock farming, about 80% of methane is produced through microbial fermentation in the rumen, while remaining 20% is emitted through the decomposition of manure (Vergé et al. 2007). However, methane is a potent greenhouse gas, and its global warming potential has been revised to 27 times that of the CO_2 (IPCC 2007). Methane production from enteric fermentation of ruminants is a major global environmental concern which contributes to 20–25% of the total anthropogenic methane emissions (Thorpe 2009). Methane emission by dairy cattle, beef lot, buffalo, and sheep and goat is 18.9, 55.9, 6.2–8.1, and 9.5 Tg per year, respectively (McMichael et al. 2007).

The methanogens share their ancestral line with bacteria but has been placed into a separated domain Archaea and the phylum Euryarchaeota. As against the bacteria, methanogens lack peptidoglycan and instead may have pseudomurein, heteropolysaccharide, or a protein in their cell wall (Balch et al. 1979). Further, they have coenzyme M_{420} (having absorbance at 420 nm) associated with hydrogenase and formate dehydrogenase, which has blue-green fluorescence at 470 nm (Rouviere and Wolfe 1988). All methanogens also need coenzyme M, which they produce themselves, or they have a nutritional need (Hobson and Stewart 1997). This coenzyme M (or 2-mercaptoethanesulfonic acid) is a methyl group carrier which produces methane. There are about 28 genera of methanogens with about 113 species. The important methanogenic groups are *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales*; among these, *Methanobacteriales* are the predominant archaeal group in the rumen (Nicholson et al. 2007). Archeal species which have been cultured are *Methanobacterium formicicum*, *Methanobacterium bryantii*, *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanomicrobium mobile*, and *Methanoculleus olentangyi*. However, the most important methanogens of the rumen are *Methanobrevibacter*, *Methanomicrobium*, and a cluster of uncultured archaea called *Thermoplasmatales* or rumen cluster C (RCC) (Janssen and Kirs 2008).

4.5.1 Rumen Protozoa

Although Grubby and Delafond described the anaerobic rumen protozoa way back in 1843, much of the knowledge signifying their role and specific functions have originated only during the past few decades. Ruminal protozoa are by far only a small proportion of total microbial population of the rumen, but they make about 40–50% of the microbial biomass inside the rumen. Protozoan population in the rumen consist mainly of the two types of ciliate anaerobes: entodinomorphids (with firm pellicles and discreet cilia generally only on peristomes) and holotrichs (flexible pellicles, covered all over with cilia). Important holotrichs are *Isotricha, Dasytricha, Buetschlia*, and *Charonina*, and some important entodinomorphs are *Entodinium*, *Diplodinium*, *Epidinium*, *Eudiplodinium*, *Metadinium*, *Polyplastron*, *Eremoplastron*, *Elytroplastron*, and *Ostracodinium*.

Depending on the enzyme profiles, protozoa may be sugar and starch utilizers (amylolytic), cellulolytic, hemicellulolytic, pectinolytic, proteolytic, or lipolytic

(Williams and Coleman 1992). Holotrichs are generally soluble sugar utilizers and stores them as starch granules for use when starch and sugar supply are limited. This ability of protozoa for engulfing and storing starch grains and other soluble sugars prevents sudden decrease in ruminal pH and saves the host animals from lactic acidosis (Williams and Coleman 1992; van Zwieten et al. 2008). Entodinomorphs remain attached to fiber and digest cellulose, hemicellulose, pectin, and starch. Fiber-degrading entodinomorphs contribute about 19–28% of the total cellulase activity of the rumen. End products of fiber and soluble carbohydrate fermentation by ciliate protozoa are the same as that of ruminal bacteria, i.e., VFAs, H_2, and CO_2. Immediately after quick engulfing of starch and soluble sugars, protozoa migrate to the fibrous mat which are retained longer in the rumen, giving sufficient time to these slow multiplying ciliates (Hook et al. 2012). Thus, both holotrichs and entodinomorphs have comparatively slow passage rate and high retention time in the rumen, the former by migrating to the ruminal mat after feeding upon soluble sugars and starch granules and the latter by attaching to the fiber particles. Fibrolytic protozoa (entodinomorphs) can feed upon ruminal bacteria and degrade microbial protein and thus affect microbial protein utilization by host animal (Belanche et al. 2012). Defaunation (elimination of protozoa from rumen) has shown to decrease ruminal methane production and improve microbial protein supply (Ivan 2009).

4.6 Rumen Fungi

The ruminal anaerobic fungi were first discovered in 1910 and were initially assumed to be flagellate protozoa (Liebetanz 1910; Braune 1913). However, during the 1970s, Orpin's study helped them be recognized as anaerobic fungi (Orpin 1975, 1976, 1977). Anaerobic rumen fungi have been classified into six genera, viz., *Neocallimastix*, *Piromyces (Piromonas)*, and *Caecomyces (Sphaeromonas*)—all with monocentric sporangia—and *Orpinomyces*, *Anaeromyces (Ruminomyces)*, and *Cyllamyces*, with polycentric sporangia. These fungi represent a small proportion of the ruminal microbes (10^3–10^5 g^{-1} rumen content in number and 3–8% of the microbial biomass) but perform important function in fiber degradation through their cellulolytic and hemicellulolytic enzymatic profiles and breaking lignocellulosic bonds. Motile fungal zoospores get attracted to the fibrous feed particles through chemotaxis, colonize the feed particles followed by development of hyphae and mycelium (splitting feed particles mechanically), and remain attached firmly to the feed particles through their rhizoids (Denman et al. 2008). This adhesion is enhanced especially by lignified substrates, and thus fungi are especially important in breaking the lignocellulosic bonds of the recalcitrant structural plant cell walls. Fungal esterase enzymes such as feruloyl esterase, p-coumaryl esterase, and acetyl esterase break lignocellulosic and ligno-hemicellulosic ester bonds and release cellulose and hemicellulose for further microbial degradation (Yue et al. 2009). The rhizoid penetrates further into the feed particles through their polysaccharide-degrading enzyme system. Akin (1989) reported that non-lignified tissues such as mesophyll, parenchyma, and phloem and the softer sclerenchyma tissue of leaf blades are degraded more extensively and completely; however, lignified xylem and sclerenchyma ring tissues in the stem are degraded partially by the fungi. Nevertheless, these breakdowns of the fibrous feed particles by the rumen fungi further enhance the site exposure and fiber degradation by fibrolytic enzymes from bacteria and protozoa.

4.7 Rumen Bacteriophage

Diversity of ruminal bacteriophages presents ample opportunity for exploring them to understand their genetic nature and interactions, lateral gene transfer, modulation of rumen fermentation, and controlling and eliminating certain ruminal microbes such as methanogenic archaea (Gilbert and Klieve 2015). Further, these are also explored for development of novel enzymes, modulation of host enzyme interactions, and application in treatment through novel branch of phage therapy. Rumen phages were first reported from bovine rumen during the 1960s (Adams et al. 1966), and soon it was established that these are common resident of the rumen affecting mostly the rumen bacteria. Rumen phages are present in significant number– 10^8–10^9 particles mL^{-1} of the ruminal fluid; however, these are the least studied microbes of the rumen. Tailed phages belonging to the families of *Myoviridae*, *Podoviridae*, and *Siphoviridae* (Ritchie et al. 1970) and phage particles from tail-less *Tectiviridae* family are some important phages observed at the ruminal fluid. In spite of their diversity in type and number in the rumen fluid, the biological properties or genetic makeup of phages are poorly understood (Gilbert and Klieve 2015). Although majority of the ruminal phages are lytic phage, only a small portion are lysogenic (temperate) phage; pseudolysogenic phages which are present simultaneously with bacteria in the same culture, in which phages multiply only on a part of the bacterial population, have also been reported (Weinbauer 2004). It has been suggested that the specificity of these bacteriophages may be utilized for control and elimination of certain harmful bacteria such as *Streptococcus bovis* and methanogenic archaea (Klieve et al. 1999; Bach et al. 2002). Diversity of ruminal phages presents ample opportunity for exploring phages to understand their genetic nature and interactions, lateral gene transfer, modulation of rumen fermentation, and controlling and eliminating certain ruminal microbes (Gilbert and Klieve 2015). Further, these are also explored for development of novel enzymes, modulation of host enzyme interactions, and application in treatment through novel branch of phage therapy.

4.8 Conclusions

The dynamic and complex ecosystem of the rumen is maintained at a constant milieu well suited for the diverse inhabitant anaerobic microbial population to grow and multiply. Temperature, pH, buffering capacity, osmotic pressure, and redox potential are the primary factors affecting the growth and activity of these microbes. Efficient microbial fermentation ensures degradation of complex structural carbohydrates and other components of feed stuffs in addition to detoxifying certain potentially toxic substances. However, methane, which is an end product of microbial fermentation inside the rumen, is a potent greenhouse gas which is a major global environmental concern. Ruminal bacteriophages are the least explored among all the rumen microbes, but they provide enormous scope for their wider applications in controlling and eliminating certain ruminal microbes and their use in treatment through the novel branch of phage therapy.

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Chapter 5
Untangling the Structure and Function of Rumen Microbes in Relation to Ruminant Health and Exploring Their Biotechnological Applications

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Abstract The gut of the higher animals is associated with various microorganisms which improves the efficiency of digestion in host animals. The most successful symbiotic relationship is found in ruminant mammals which provide the space and nutrients to various microbes (anaerobic bacteria, methanogens, anaerobic fungi, protozoa, and bacteriophages) in their rumen. The digestive track in ruminants performs the following functions: periodic eructation, rumination, digestion of feed, use of non-protein and protein nitrogen, fat digestion, and vitamin production. During the rumen microbiome maturation, various factors (such as host species, diets, geographical location, feed supplements (probiotics), antibiotic treatments, etc.) are known to alter and affect the rumen microbial profiles. The rumen microbiome influences the growth and health performance of the ruminants and also affects the various fermentation parameters. The interaction of rumen microbes with the host were studied to improve the development and the health of the ruminants by microbial manipulating approaches. Ruminant animals lack the enzymes involved in the digestion of cellulose (plant cell wall component), and therefore depend on the rumen microbes which produce the hydrolytic enzymes and provide the microbial environment for the degradation of the plant-based feeds/substrate. The development of various omics approaches has provided the information on rumen microbiome and their secondary metabolites and also their effect on immune response and gastrointestinal activities of ruminants. Therefore, better understanding of structural and functional diversity of rumen microbiota can improve the growth and health performances of host. Apart from these, rumen microbes are widely used as a source of renewable energy (biogas production) in agriculture (bio-fertilizers) and industries (rumen microbial enzymes as xylanase, endoglucanase, and esterase, etc.). They are extensively used for production of various rumen-based products (prebiotic and probiotic) which improve the rumen

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_5

microbial fermentation and host immunity and prevent the colonization of pathogenic microbes.

Keywords Gut microbiota · Ruminant mammals · Rumen microbiome · Hungate technique · Gnotobiotic · Planktonic bacteria

5.1 Introduction

Microorganisms are one of the essential elements of the biosphere which play a key role in stabilizing the living system. They are ubiquitous in nature and present in almost all habitats on earth. During the path of evolution, microbes symbiotically associated with higher organisms leading to the several benefits such as photosynthesis, aerobic digestion (endosymbiotic theory), accumulation of nutrients (VAM fungi and plants), etc. In addition, the digestive system of higher animals becomes the home of several microorganisms thereby enhancing the digestive efficiency of the host. The composition of gut microbiota is highly complexed and fluctuates in response to various biotic and abiotic factors. They work in highly coordinated fashion to make the overall digestive system more efficient. Although they are termed as "gut microbes," their function is not only restricted to digestion. Recently, studies have evidenced a critical role of gut microbes in human health and disease by energy extraction from food and nutrient harvest, changing appetite signaling, defending against pathogens by competitive exclusion, and producing antimicrobial substances (Wang et al. 2017). The best example of symbiotic association is in ruminant mammals which contain microbes in their rumen. There are approximately 200 extant (currently living) species of ruminants which belong to the following families: Antilocapridae, Giraffidae, Bovidae, Cervidae, Moschidae, and Tragulidae (Hassanin and Douzery 2003). Among these, Bovidae is the diverse family that consists of 143 known species including antelopes (blackbuck, *Antilope cervicapra*; roan antelope, *Hippotragus equinus*), cattle (*Bos taurus*), sheep (*Ovis aries*), goats (*Capra hircus*), water buffalo (*Bubalus bubalis*), African buffalo (*Syncerus caffer*), bison (*Bison bison*), and yak (*B. grunniens* and *B. mutus*) (Heller et al. 2013). They are distributed across diverse habitats including different ecological environments from deserts to rainforests, tropical to arctic regions, and plains to plateaus. Moreover, they exhibit the unique anatomical features such as multi-chambered stomachs and osseous cranial appendage (headgear). The digestion of fibrous plant materials is more efficiently enabled in the rumen and omasum in ruminant animals compared to other herbivores (such as horses) (Russell and Rychlik 2001). The ruminants are successfully procuring for energy extraction from food and possess specialized teeth and substantial variation in body size. Moreover, they play a main role in human civilization due to several domestic animals (buffalo, cattle, goat, sheep, yak, etc.) which are important for the modern human diet and nutrient utilization. The rumen microbiome is presumed to influence the fermentation parameters, health, and growth performance of host animals (Malmuthuge and Guan 2017). Ruminants, like other vertebrates, are unable to digest plant materials directly due to a lack of enzymes capable of degrading the plant cell walls' principal constituent (cellulose). As a result of the development of a complete collection of enzymes needed to cleave the various chemical bonds inside the cell wall, colonization on feed particles, and synergism between different species of ruminants, ruminant animals create a complex and sophisticated microbial environment for plant cell wall degradation (Wang and McAllister 2002). Therefore, untangling the structural and functional diversity of rumen microbes to improve the health and growth performance, ruminants are tremendously important for scientific research and their applications in human societies.

5.2 Historical Perspective of Rumen Microbiology

The rumen microbial population (also known as rumen microbiome) are microorganisms that are adapted to grow in an anaerobic (absence of oxygen) environment. In 1860, Louis Pasteur defined that some microbes could adapt, survive, and grow in an anaerobic environment through the fermentation process. During the twentieth century, Bryant (1997) has completely described the history of evolution of gut microbes and also contributed much more for the development of the gut microbiology. Until the anaerobic chamber was created, inoculation and incubation of microbes in an anaerobic environment were unsuccessful (Aranki et al. 1969). However, the roll tube anaerobic technique to strictly (obligatory) cultivate anaerobes was first developed by Robert E. Hungate, who was considered as the father of rumen microbiology. This technique is also known as "Hungate technique" (Hungate and Macy 1973) which has widely been used for the cultivation of anaerobes. The axenic cultivation, identification, and enumeration of each kind of organism, relative fermentation rates, and turnover rates of substrate in their natural microbial habitats (such as rumen) have been hardly overemphasized (Hungate 1960).

During the second half of the twentieth century, the impetus technologies have been derived from the following three main sources for most important research advances in rumen microbial ecology: the creation of anaerobic techniques and their application to investigate the rumen microbial environment (Hungate and Macy 1973), the use of rodent experimental models to establish inter-relationship between microbes and their animal hosts (Raibaud et al. 1980), and the development of gnotobiotics by which both germ-free and defined-microflora animals could be normally derived and maintained to study host microbial relationship (Gordon and Pesti 1971).

5.3 Rumen Microbial Ecosystem

The rumen is a diverse and dense microbial ecosystem that includes a symbiotic population of obligatory anaerobic microbes (such as bacteria, protozoa, fungi, etc.) from all major domains of life (Kim et al. 2011). They are well adapted to establish, survive, and proliferate in an anaerobic condition by using the fermentation process. Ruminant growth and development are thought to be aided by the microbial fermentation process in the rumen. Upon ingestion of feeds, the ruminants can digest them to various extents by the synchronous activities (i.e., synergism and antagonism) of highly diversified rumen microbial populations consisting of 50 bacterial genera ($10^{10–11}$ CFU/ml), archaea ($10^{8–9}$ cells/ml), 25 ciliate protozoa ($10^{4–6}$ cells/ml), six anaerobic fungal genera ($10^{3–6}$ zoospores/ml), and bacteriophages (10^8–10^9 PFU/ml) (Kamra 2005; Matthews et al. 2019). Generally, the rumen comprises 8–15% (w/w) content of the total animal and has about 10–13% content of dry matter (Choudhury et al. 2015). Usually, the regulated temperature is between 38 and 41 °C. This microbial ecosystem is specially buffered to the narrow range of pH (5.5 and 6.9) which helps the animal stabilize the ecosystem without any disturbance in feed and water intake, from incoming microbial pollutants into the rumen. In comparison to domestic animals (such as cattle, sheep, and goat), the rumen microbial ecosystem of buffalo and wild ruminants is very poorly studied.

5.4 Host-Rumen Microbial Interactions

Ruminant metabolic disorders and health are related to the microbial population composition and functions in the rumen. The host-rumen microbial interactions have primarily been investigated in order to improve ruminant development and health. Furthermore, the majority of host-rumen microbial interaction studies connect rumen microbes to host characteristics (such as feed efficiency, reducing methane emission, milk production, susceptibility to metabolic disorders, etc.) (Malmuthuge and Guan 2017). Studies on the contribution of the rumen microbial populations directly toward the ruminant's nutrition provided only a general knowledge on the metabolic processes within the rumen. But, still, there is yet more to be studied about the individual microbe that colonizes the organs of the ruminant. However, it is likely to contribute approximately 60% bacteria (Henderson et al. 2015), only ~1% archaea (Poulsen et al. 2013), less than 8–12% anaerobic fungi (Matthews et al. 2019), and ciliate protozoa up to half (Lourenço et al. 2010) of the total microbial biomass in ruminants in relation to the digestion of feed particles.

To alter the host's growth efficiency and prevent disease, the rumen microbial composition will be manipulated in the adult and young ruminants through the nutritional (dietary) intervention (Uyeno et al. 2015). The nutritional intervention in early life (3 months old) of goat kids has been shown to change the rumen microbial composition and affect the host traits (feed and supplement intakes, body weight gain, reducing methane emission, rumen fermentation profile, volatile fatty acids production, etc.) in the post-weaning period (Abecia et al. 2013). Changes in the early rumen microbiota composition may have an impact on rumen microbial succession and host traits; additionally, microbial manipulation in young ruminants may be more successful than manipulation in adult ruminants (Malmuthuge and Guan 2017). As a result, it's critical to gain a better understanding of the host-rumen microbial relationship early in life in order to develop successful microbial manipulation strategies with long-term effects on various ruminants.

The epimural (rumen epithelial adherent) microbial group, which maintains near associations with the host, is more complex than the material- or fluid-associated rumen microbial community, according to studies, and its composition can be influenced by dietary intervention (Chen et al. 2011). The density of the epimural microbial population is linked to improvements in the host's gene expression and the prevention of subacute ruminal acidosis (Chen et al. 2012). Even though the functional role of epimural microbial community is hypothesized to direct involvement in the active oxygen scavenging performance, hydrolysis of urea, tissue recycling, and amino acid metabolism (Wetzels et al. 2017), this population has a very poor understanding. As a result, future research into host-microbial interactions must include the rumen epimural microbial community. Since the researcher's primary emphasis is on rumen microbial aspects, there is a lack of knowledge about the host's role in controlling rumen microbiota composition, which could lead to inconsistency when the same nutritional intervention is used. Furthermore, studies suggested that the host influences the restoration of original status in the rumen microbial composition after dietary intervention (Uyeno et al. 2015). Some animals restore the originality faster than others when rumen contents are exchanged between two ruminant livestock species (Weimer et al. 2010). Therefore, the development of microbial manipulation methods must also address thoroughly the host mechanisms that may impact the rumen microbial composition.

5.5 Rumen Microbial Diversity

5.5.1 Functional Diversity of Rumen Microbes

Due to the lack of enzyme production by ruminants, rumen microbes are forced to play an important role in the digestion and fermentation of the feed's primary components. As a result, a better understanding of rumen microbial function and population dynamics could lead to novel cost-effective strategies for ruminant survival and efficiency. The ruminal microbiota is characterized by a high population density, a wide range of species, and a complex interplay with the host. Historically, understanding the rumen microbial ecology based on the culture-dependent techniques was pioneered by Robert E. Hungate (Krause et al. 2013). The classical culture approaches were implemented to better comprehend the rumen microbial environment and functions related to the host nutrition and sustainable food production before the swift rise of the high-throughput multiple omics approaches. The rumen microbial population is extremely diverse, with hundreds of different bacterial and archaeal species, as well as numerous anaerobic fungi and ciliate protozoa species.

5.5.2 Bacteria

Bacteria, which are strictly anaerobes, are the most common microbes found in the rumen. They make up more than 95% of the rumen microbial population, with a number of 10^{10}–10^{11} CFU/g of rumen content (Choudhury et al. 2015). In general, it is assumed that culture-based techniques have identified only 10–20% of the bacterial species present within the rumen (Krause et al. 2013). The bacterial population has been divided into four major groups based on their association with the different micro-environments found in the rumen: solid-adherent bacteria (bound to feed particles), planktonic bacteria (free-living in the liquid and detached from the feed particles), epimural bacteria (rumen epithelial-adherent), and eukaryote-associated bacteria (attached to eukaryotes in the rumen, such as protozoa and fungi) (De Mulder et al. 2017). In ruminants (dairy cattle, sheep, and steer), epimural bacteria are the most distinct from other bacteria and can have a greater impact on the host's metabolic activities (Liu et al. 2015). They are frequently facultative anaerobes and have some specific functions such as urea hydrolysis, maintain rumen anaerobiosis by oxygen scavenging and epithelial tissue recycling, and may not help in the digestion and fermentation of the rumen (Nagaraja 2016). Despite this, due to the conventional low-throughput method of identifying bacterial 16S rRNA clone libraries and DNA fingerprinting, knowledge on the number of epimural bacterial communities is still limited.

The rumen bacteria are further classified into based on their substrate (cellulose, hemicellulose, pectin, starch, and amino acids) preference, energy requirements, and use of fermentation end-products. The main cellulolytic (cellulose-degrading) bacteria present in the rumen are *Fibrobacter succinogenes*, *Ruminococcus albus*, and *R. flavefaciens* (Koike and Kobayashi 2001). The most abundant ruminal bacteria which also exhibit the degradation of hemicelluloses (glucomannan, xylans, and xyloglucan) are *Butyrivibrio fibrisolvens*, *Prevotella ruminocola*, *R. albus*, and *R. flavefaciens* (Puniya et al. 2015). In comparison, certain anaerobic bacteria (such as *B. fibrisolvens*, *Lachnospira multiparus*, *P. ruminicola*, and *Succinivibrio dextrinosolvens*) get their energy from pectin degradation by converting oligogalacturonides and developing significant amounts of acetate in bovine metabolism (Dusková and Marounek 2001). Many ruminant animals are fed a grain-based diet, which adds a significant amount of starch (as a substrate) into the rumen for microbial fermentation. The rumen bacteria such as *B. fibrisolvens*, *Clostridium* spp., *F. succinogenes*, *P. ruminocola*, *Ruminobacter amylophilus*, *Selenomonas ruminantium*, *Streptococcus bovis*, and *Succinimonas amylolytica* play a crucial role in the fermentation of starch (Puniya et al. 2015). However, the study indicated the presence of additional amino acid-fermenting bacteria, capable of amino acid deamination within the rumen and causing rapid production of ammonia and deriving adenosine triphosphate (ATP) for energy source (Bento et al. 2015).

5.5.3 Archaea

The strictest anaerobes, the rumen archaea are mainly made up of methane-producing bacteria in the rumen known as methanogens (Janssen and Kirs 2008). They are found in the range of about 10^8–10^9 cells/g of rumen content and accounting for $<4\%$ of the total rumen microbial community (Matthews et al. 2019). Because of their use as fermentation end products (as substrates), they are present at the bottom of the trophic chain (Morgavi et al. 2010). So far, only thirteen species of methanogens have been isolated as pure cultures from ruminant rumens: *Methanobacterium beijingense*, *Methanobacterium bryantii*, *Methanobacterium formicicum*, *Methanobrevibacter boviskoreani*, *Methanobrevibacter millerae*, *Methanobrevibacter olleyae*, *Methanobrevibacter ruminantium*, *Methanoculleus bourgensis*, *Methanoculleus marisnigri*, *Methanoculleus olentangyi*, *Methanomicrobium mobile*, *Methanosarcina barkeri* and *Methanosarcina mazei* (Patra et al. 2017).

Most of the methanogens use hydrogen gas (H_2 or formate) as an electron-donating reductant to reduce carbon dioxide (CO_2) to methane (CH_4), whereas other species may oxidize methyl groups to CO_2 before reducing to CH_4. Even though few archaeal species can effectively dissimilate acetate to CO_2 and CH_4, ruminant animals do not convert ruminal acetate to CH4. This is most likely because the rate at which ruminal contents move through the rumen is faster than the rate at which acetate-utilizing (aceticlastic) methanogens expand (Janssen and Kirs 2008). Rumen methanogenesis (the production of methane by methanogens) results in a net energy loss of about 2–12%, which is no longer usable for the animal's development, maintenance, lactation, or pregnancy. Long-term reduction of methane emissions in ruminants may be achieved by manipulating the ruminant's diet, using anti-methanogens (such as bromochloromethane), and using an invasive species of macroalgae (*Asparagopsis taxiformis*) (Matthews et al. 2019).

5.5.4 Ciliate Protozoa

Protozoa, which include ciliates and flagellates, are strict anaerobes that depend on their hosts for nutrition. Ciliate protozoa are more common in rumen fluid, with concentrations of 10^4–10^6 cells/ml, and account for 30–40% of total fiber digestion (Matthews et al. 2019). They are also active in lipid hydrolysis, and their hydrosomes have the ability to generate hydrogen (Tymensen et al. 2012). *Entodinium* is the most popular protozoan that degrades starch quickly, engulfing and transforming it to an iodophilic storage polymer in high-grain diets (Matthews et al. 2019). Protozoa-associated methanogens are responsible for about 37% of methanogenesis in ruminants' rumens, and they may be the rumen's most active endosymbiotic methanogen populations (Finlay et al. 1994). The reduction in methane production may be explained by defaunation, a process that prevents protozoa from growing or being removed from the rumen (Hook et al. 2010). Protozoa engulf bacteria and other small microbes and particles in the rumen, as well as larger molecules like proteins and carbohydrates, so their presence in the rumen microbiome may have both positive and negative consequences (Bach et al. 2005). They vigorously consume the bacteria as a protein source, as well as acting as a stabilizer for the rumen fermentation (Denton et al. 2015).

5.5.5 Anaerobic Fungi

Rumen fungi are anaerobic and belong to the *Neocallimastigomycetes* class, which includes the genera *Anaeromyces*, *Buwchfawromyces*, *Caecomyces*, *Cyllamyces*, *Neocallimastix*, *Oontomyces*, *Orpinomyces*, and *Piromyces* (Kittelmann et al. 2012). Since anaerobic fungi produce H_2 during the initial degradation of plant tissues, which can be used as a fuel for other communities' degradation mechanisms, they can have an effect on the rest of the microbial population. Rumen anaerobic fungi colonize plant cell walls and account for 8–12% of total rumen microbial biomass, though this proportion varies greatly depending on diet (Matthews et al. 2019). Fungi produce a lot of cellulases and hemicellulases, and they also have the ability to break down xylan because they proficiently produce xylanases (Akin and Borneman 1990). Fungi appear to initiate the feed breakdown process, meaning that anaerobic fungi are critical for pasture-fed ruminant feed utilization quality, as well as animal growth and development (Kittelmann et al. 2012).

5.5.6 Bacteriophages

Bacteriophages are most abundant obligate bacterial pathogens that live in dense colonies in the rumen, averaging 10^7–10^9 particles/gram of digesta (Matthews et al. 2019). The rumen virome is the population of bacteriophages and viruses present in the rumen (Ross et al. 2013). The large number of rumen bacteriophages indicates that they may be significant in maintaining the rumen system's equilibrium, but little is known about the rumen virome's effect on the system it lives in. The three most common bacterial phyla (*Bacteroidetes*, *Firmicutes*, and *Proteobacteria*) were found to be associated with the majority of viruses found in the rumen (Berg Miller et al. 2012). The development of plaques in otherwise confluent layers of bacterial growth suggests effective bacteriophage infection and replication, so culture-based techniques have also been used to study ruminal bacteriophage populations (Gilbert et al.

2017). These culture-based techniques capture viable phages that can efficiently infect and replicate in the bacterial strains used in the sample, rather than the viral variety of the rumen source material.

5.6 Structural Diversity of Rumen Microbes

The limited functional knowledge on the uncultured microbes does not support the understanding of significance of microbial populations in rumen. However, the culture-independent techniques for a comprehensive view at the microbiota of the rumen evolved in the early 1990s. Our ability to classify rumen microbial communities and investigate their dynamic relationships between structural diversity and ecosystem functions has been redefined by high-throughput DNA sequencing technologies and the availability of multi-omics data (McCann et al. 2014). The nucleic acid-based techniques used to study the relation of structural and functional diversity of rumen microbes are polymerase chain reaction (PCR) and microbial community fingerprinting strategies such as denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphism (RFLP), and automated ribosomal intergenic spacer analysis (ARISA).

Shotgun metagenome sequencing, in general, aids in evaluating total microbial diversity and detecting relative anaerobic microbial abundance in rumen fluid and feces samples (Zinicola et al. 2015). In comparison to shotgun sequencing, amplicon sequencing of the 16S rRNA gene is the most widely used approach to research the rumen microbial population. Furthermore, 16S rRNA gene sequencing only provides information on bacterial populations, not fungal, protozoa, or viral populations, all of which are essential in rumen fermentation. Despite the fact that several metagenomic and metatranscriptomic studies on the rumen microbiome have been published, the number of these studies is still small. This is due to the fact that widespread use of these techniques is still constrained by higher costs, as well as the need for bioinformatics software and expertise to manage large datasets and interpret them.

5.6.1 Bacteria

The conserved 16S rRNA gene amplicons were first sequenced as a strong phylogenic marker to research the abundance of bacterial populations in the rumen. Later, competitive PCR (cPCR) assays for rumen cellulolytic bacteria (*F. succinogenes*, *R. albus*, and *R. flavefaciens*) enumeration were developed (Koike and Kobayashi 2001). Using 16S rRNA sequencing, PCR-DGGE, and quantitative real-time PCR (qRT-PCR) study, Chen et al. (2011) found that dietary shifts from a high-forage to a high-grain diet affect the total rumen epimural bacterial population density and diversity in the bovine rumen. The high-throughput pyrosequencing has been used for investigating the changes in epimural bacterial diversity and richness in high-grain diet feeding ruminants (cattle, sheep, goat, and heifer) (Petri et al. 2013).

5.6.2 Archaea

The methyl coenzyme M reductase (mcrA) gene is used as an alternative phylogenetic tool to 16S rRNA in the specific detection and identification of methanogen populations (Luton et al. 2002). The archaeal diversity in anaerobic digesters was discovered using single-strand conformation polymorphism (SSCP) analysis of the 16S rDNA V3 region, and the most frequent sequences were close to *Methanosaeta concilii* (84%) and *Methanobacterium* clade (73%) of digesters, respectively, and the other sequences were matched to *Methanobacteriales* and *Methanomicrobiales* families (Leclerc et al. 2004). A variety of different methanogen species have been identified by analyzing the archaeal small subunit ribosomal RNA (ssrRNA) genes from the rumen samples of ruminants and suggested that the majority of the known methanogens fall into three major groups: *Methanobrevibacter*, *Methanomicrobium*, and uncultured rumen cluster C (RCC) (Janssen and Kirs 2008). The rRNA-targeted fluorescence in situ hybridization (FISH) has been used to analyze the rumen archaeal community and their activities (Kumar et al. 2011). According to the data from global meta-analysis, approximately 90% of rumen methanogens belong to the genera *Methanobrevibacter* (63.2%), followed by *Methanosphaera* (9.8%), *Methanomicrobium* (7.7%), *Thermoplasma* (7.4%), and *Methanobacterium* (1.2%) (Patra et al. 2017).

5.6.3 Ciliate Protozoa

Since protozoa can be fragile and inconspicuous, and determining whether a given morphological feature is distinct or not can be difficult, accurately detecting or identifying many protozoan species through microscopic analysis of morphological features remains difficult and time-consuming. The small subunit ribosomal RNA (SSU-rRNA) gene, also known as 16S rRNA in prokaryotes and 18S rRNA in eukaryotes, is often used as a molecular marker to distinguish morphologically related species, recognize phylogenetic relationships, and elucidate diversity. The protozoal diversity in rumen samples has been extensively studied using PCR-sequencing methods (Karnati et al. 2003). In addition, PCR–DGGE has been used to profile protozoal groups in the rumen (Sylvester et al. 2005). There is a strong positive interaction between protozoal numbers and methane emissions (Morgavi et al. 2010). They discovered that methane emissions increased as protozoal abundance increased using qPCR with DNA derived from rumen contents and that protozoa had the greatest association with methane emissions in comparison to other domains (Wallace et al. 2014).

5.6.4 Anaerobic Fungi

Anaerobic fungi are the only known fungi to maintain cell wall-associated multi-enzyme extracellular complexes (cellu1losomes) and are thought to have developed through horizontal gene transfer from bacteria (Garcia-Vallvé et al. 2000). Metagenomic approaches may be able to determine the degree and source of horizontal gene transfer. The life cycle contrast between low zoospore numbers and high anaerobic fungi vegetative biomass, as well as the paucity or absence of zoosporogenesis seen in some polycentric axenic cultures, has been resolved in recent years by molecular quantification using the real-time PCR process (Hess et al. 2020). On the other hand, the real-time PCR approach for quantifying fungal biomass has its own set of challenges, such as varying amounts of fungal biomass generated by monocentric, polycentric, and bulbous genera in relation to DNA content (Edwards et al. 2008). It's incredibly difficult to turn quantitative real-time PCR estimates into fungal biomass because of this. The number of ITS1 spacer regions used to estimate the abundance of anaerobic fungi appears to be taxon independent (Mura et al. 2019). However, it is uncertain if all anaerobic fungi have the same number of copies of the ribosomal RNA (*rrn*) operon.

5.6.5 Bacteriophages

Bacteriophages are the most common rumen viruses, and they dominate public databases, making their detection based on genetic homology easier. Understanding virus populations requires the use of sequencing, and we now have a plethora of resources at our disposal that make creating virus-specific sequence datasets much easier. Due to the lack of a universally conserved gene in all viral organisms, shotgun metagenomics is the only way to research the viral species (Wylie et al. 2015). However, another application of metagenomics to the study of rumen virus populations is examining at the clustered regularly interspaced short palindromic repeats (CRISPR) sequences. The presence of CRISPR and CRISPR-associated proteins in the microbial population can be used to detect bacteriophage-host interaction (Berg Miller et al. 2012). In certain bacteria and archaea, these genes are essential for adaptive immunity, which enables the organism to react and eradicate invading genetic material. The new DNA is inserted as new spacers into the host CRISPR locus until infected with these invading genetic materials, ultimately encoding a special spacer sequence. According to metagenomic studies, the rumen virome is dominated by *Siphoviridae* (32–36%), *Myoviridae* (24–32%), and *Podoviridae* (12–16%), all bacteriophage families belonging to the order

Caudovirales (Gilbert et al. 2020). As an alternative molecular method for enumerating and differentiating rumen bacteriophage population, the use of pulsed field gel electrophoresis (PFGE) to create a genetic fingerprint of bacteriophage population based on their genome length was introduced (Swain et al. 1996). The PFGE technique was also used to show that the ruminal bacteriophage populations may have a diverse composition and be highly dynamic.

5.7 Ruminant Physiology

The ruminant's (cattle, sheep, goats, buffalos, deer, giraffes, and antelopes) digestive system consists of the mouth, esophagus, four compartments of the stomach, small intestine, and large intestine. The esophagus is a muscular tube connecting the mouth and stomach. Rumen (paunch), reticulum (honeycomb), omasum (manyplies), and abomasum (true stomach) are the four compartments in a ruminant's stomach (Fig. 5.1). It constitutes an appropriate environment to develop and establish the greater diversity of microbes including bacteria, fungi, and protozoa (Karasov and Douglas 2013). The rumen, located on the animal's left side, is the stomach's largest compartment, consisting of the dorsal sac, ventral sac, caudodorsal sac, and caudoventral sac. Depending on the size of the animal, it can carry up to 25 gallons of material. Because of its size, the rumen serves as a feed storage tank. Aside from being used for storage, it's often used as a fermentation vat because the atmosphere is conducive to microbial development. Within the rumen, these microbes ferment or digest the feed, producing volatile fatty acids (VFAs: acetic acid, propionic acid, and butyric acid) as an energy source. Amino acids and vitamins (B and K) are also provided by the rumen microbes (B and K).

The small intestine is divided into three parts (duodenum, jejunum, and ileum) and is approximately 20 times the length of the animal. It completes most of the digestive process by the enzyme secretions from the pancreas and gall bladder and

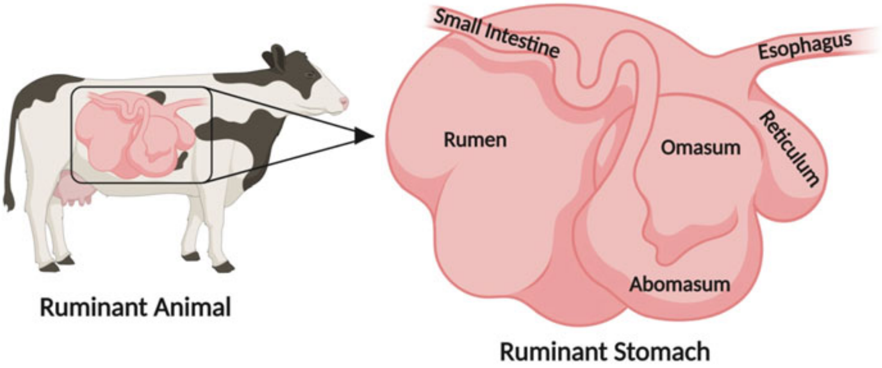

Fig. 5.1 Basic morphology of the ruminant's stomach of digestive system

absorbs the nutrients through villi. Thereafter, the nutrients penetrate from the intestinal villi into the bloodstream and lymphatic systems. The cecum is the large junction between the small and large intestines. It helps in the digestion of previously undigested fibers, but its exact function is unknown. The large intestine is the final segment of the digestive tract, where undigested and unabsorbed food is removed as waste (excreta). It mainly absorbs water after microbial digestion. The digestive tract in the ruminants is attached to the digestive glands (such as pancreas, salivary glands, gall bladder, and liver) which secrete the digestive enzymes (Membrive 2016). The main functions of digestive tract in the ruminants are the periodic eructation (belching of rumen gas), rumination (cud chewing), saliva production for buffering rumen pH, vomiting, movement and digestion of feed, use of protein and non-protein nitrogen in the rumen, vitamin production, and fat digestion.

5.7.1 Rumen Fermentation Pathways

The rumen is a fermentation vat in the ruminants that houses a highly diverse microbial population including bacteria, protozoa, fungi, etc. Rumen microbes play an important part in improving the animal health and immune function. These microbes also enable the animal to ferment and breakdown the lignocellulosic plant materials into the useable energy for the host animal. Anaerobic fermentation can make up to 70% of total dietary energy in the case of large herbivores (Flint and Bayer 2008). The herbivorous ruminant animals ingest the mixture of plants that are usually moistened with saliva secreted from the salivary glands and chewed completely in the mouth. After mastication, the food is ingested into rumen, where it is ruminated and then, subjected to the enzymatic digestion by the action of symbiotic ruminal microbes. The primary role of the ruminant's digestive system is to provide a constant supply of carbohydrates, proteins, lipids, and vitamins from the feed intake to the animal after the physical, chemical, and enzymatic digestion (Fig. 5.2).

In the reticulorumen (main digestive compartment), the ingested feedstuffs are exposed to a diverse microbial population that symbiotically convert plant cell wall components to carbohydrates and sugars via fermentation and digestion. The carbohydrates provide the energy from the ruminant diets to both ruminal microbes and host animals and also create the ruminal physical environment where the microbes can grow. A large number of cellulolytic, hemicellulolytic, and pectinolytic enzymes degrade the plant's structural carbohydrates (such as cellulose, hemicellulose, and pectin). Microbial fermentation process is not limited to structural carbohydrates but also includes non-structural carbohydrates and proteins that are digested first in the rumen. The non-structural carbohydrates (like starch and soluble sugars) can be rapidly utilized by ruminal microbes before it can be hydrolyzed enzymatically and absorbed either in the abomasum or small intestine.

The large amount of glucose (six carbon atoms) production from the carbohydrates is one of the rumen's fermentative digestion's intermediate stages. Owing to

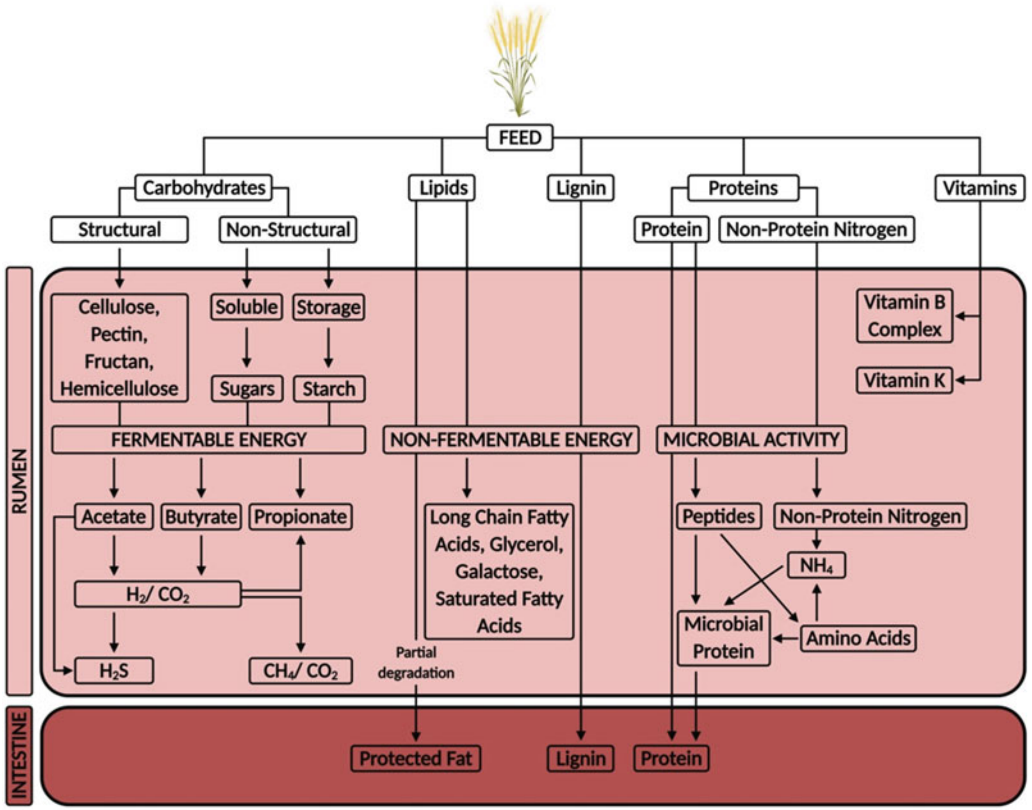

Fig. 5.2 Feed digestion pathway in ruminants' digestive system

the rumen microbes' rapid utilization of glucose, it is not readily accessible to the animal as an energy source. The anaerobic bacteria reduce glucose and produce adenosine triphosphate (ATP) which can be used as a source of energy by the animal via oxidative metabolism of VFAs in the ruminal environment. The rumen microbes ferment the sugars to produce VFAs and a huge amount of gases [comprising of methane (CH_4), carbon dioxide (CO_2), hydrogen sulfide (H_2S), and molecular hydrogen (H_2)] as the main products which are removed continuously from the rumen. The VFAs are considered as the greatest energy source for the ruminants. The VFAs produced are acetic acid, propionic acid, and butyric acid, which are transformed rapidly into their saturated or ionized forms (acetate, propionate, and butyrate, respectively) in the rumen. Acetate (two-carbon atoms) is the most abundantly produced VFA, followed by propionate (three-carbon atoms) and butyrate (four-carbon atoms). These VFAs provide 50–70% of ruminant's energy and are then absorbed through the rumen wall and transported to the liver. Typically, pH of the rumen ranges from 6.5 to 6.8. Increased VFA production results in a strongly acidic environment (pH 5.5), causing a shift in the microbial community structure and possibly lowering feed digestibility. The ruminant's ability to buffer the ruminal pH and absorb these acids is harmed by the consistent production of copious amounts of lactic acid (or lactate as a by-product of starch fermentation) combined with a higher production of VFAs. This acidic environment leads to ruminal acidosis and ulceration (Owens and Basalan 2016).

The degradable intake protein (DIP) is broken down by the rumen microbes into ammonia (NH_3, as a non-protein nitrogen compound), amino acids, and peptides, all of which are used by the microbes for protein synthesis and energy from carbohydrate digestion in order to facilitate growth and reproduction. Excess ammonia is absorbed from the rumen and converted to urea in the liver before being released back into the bloodstream. When the available ammonia exceeds the requirement, it is further used by microbes for protein synthesis. Undegradable intake protein (UIP, also known as rumen bypass protein) in feedstuffs is not degraded by the rumen. The UIP and microbial protein bypass the rumen and flow down for further digestion and absorption through the omasum to the abomasum and small intestine. To break down proteins and fats, the abomasum produces hydrochloric acid (HCl) and digestive enzymes (such as pepsin) and receives pancreas-secreted digestive enzymes (such as pancreatic lipase). These secretions help absorb the proteins and fats in the small intestine after they have escaped from the rumen microbial action. Generally, the low pH (3.5–4.0) in the abomasum is due to the production of HCl and digestive enzymes, and it secretes mucous to protect from acid damage. Because the rumen microbes synthesize all of the B and K complex vitamins in sufficient amounts for the ruminant's growth and maintenance, these vitamins are not required to be supplemented.

5.8 Factors Influencing the Rumen Microbial Population

The scientific understanding of the factors that have an impact on the establishment and development of the rumen microbial population could lead to novel strategies and opportunities for manipulating their composition in order to improve long-term host health and performance traits. Ruminant maturity is inextricably related to the rumen microbes' stability (Jami et al. 2013). Various factors have been identified to change and influence rumen microbial profiles during rumen microbiome maturation. The factors [such as host species, diets, geographical location, feed supplements (probiotics), antibiotic treatments, etc.] known to influence the ruminal microbiome composition have been well studied in the ruminants (Matthews et al. 2019). A better understanding of these factors and their effects on livestock production could be critical for developing effective strategies to improve rumen microbial composition as well as the efficiency of the ruminant host.

5.8.1 Host Effect

Ruminant hosts and ruminal microbes have a symbiotic relationship that aids in the digestion of dietary fibers in the rumen. The balance of a stable rumen environment and microbial fermentation is critical to this symbiosis. The digestive enzymes required for anaerobic fermentation of ruminant feeds, as well as the synthesis of proteins and vitamins for host health, are provided by rumen microbes (Millen et al. 2016). As a result, the rumen environment must be conducive to microbial survival and growth. According to Jami et al. (2013), changes in rumen microbiome composition are linked to ruminant age and growth. In mammals, the influence of host genetics on the formation of individual gut microbial composition has been demonstrated using mouse models (Buhnik-Rosenblau et al. 2011), and the same kind of interaction may also occur in the ruminants. The age and growth of the host, in addition to genetics, are major determinants of the rumen microbial community structure (Jami et al. 2013).

Recent developments in metagenomics methods have enhanced the study of microbial genes linked to host phenotypes. Paz et al. (2016) have suggested that under the same dietary conditions, the rumen bacterial community composition of Holstein and Jersey cows differs significantly, with no such difference due to sampling method. However, as reported by Roehe et al. (2016), the host genetics influences the rumen microbial composition and abundance of methanogens thereby affecting the methane production. The composition of rumen microbiota in the hybrid offspring [*sika deer* ♀ × *elk* ♂ (SEH) and *elk* ♀ × *sika deer* ♂ (ESH)] differed from that in their parents [sika deer (*Cervus nippon*) and elk (*Cervus elaphus*)], suggesting the considerable effect of host genetics on the rumen microbiome due to vertical transmission (Li et al. 2016). The host genetics, according to these studies, has a significant impact on the rumen microbiome composition and metabolites in hybrid animals. Further research revealed a connection between host physiology and the rumen microbiome. It was revealed that the smaller rumens and shorter digesta retention time in the rumen of sheep attributed to between-animal differences with respect to the production of methane among the constant diet-fed animals (Goopy et al. 2014). The retention time of rumen digesta and rumen volume are directly associated with rumen microbial variation, and milk protein and fat, dry matter intake, and residual feed intake have all been shown to be heritable host traits (Sasson et al. 2017).

5.8.2 Diet Effect

The composition and activities of the rumen microbial community in domesticated ruminants are known to be influenced by the diet (Tajima et al. 2001; Wright et al. 2004). Ruminant animals are subject to a variety of dietary and nutritional changes during their lives. During the pre-ruminant to ruminant transition, the animals' diets change from milk to a few solids, and they can also ruminate at condensed pellet starters. Ruminants begin to experience large dietary shifts as their rumen develops, and they are greatly affected by changes in the external environment and nutrition (Diao et al. 2019). The forage-based diet is dominated by the phyla *Fibrobacteres*, *Bacteroidetes*, and *Firmicutes*. The bacterial population (*Megasphaera elsdenii*, *Prevotella bryantii*, *Selenomonas ruminantium*, and *Streptococcus bovis*) grows during the transition from a high-forage to a high-concentrate diet. These bacterial species have been reported as lactate-utilizing bacteria to stabilize the rumen pH (Chaucheyras-Durand et al. 2012). However, the population of fibrolytic bacteria (such as *Butyrivibrio fibrisolvens* and *Fibrobacter succinogenes*) has decreased due to an increase in dietary concentrate, thus displaying bacterial variations dependent on substrate priority.

Faniyi et al. (2019) recently reported that the transition from a high-forage to a high-concentrate diet causes a significant shift in rumen microbial communities during the weaning transition period. It is well-known that the diet has an impact on fermentation characteristics and the rumen content-related microbial population structure (Russell and Rychlik 2001). The feeding of high-grain diets resulted in an increase in the rumen's dietary starch level, which causes ruminal acidosis. The abundance of an amylolytic bacterium (*Streptococcus bovis*) is high for conversion of starch into sugar, and it is attributed to a rapid increase in level of glucose and the loss of protozoa in the rumen as a result of the more acidic environment caused by high-grain diets. A decrease in ruminal pH is quite common after the consumption of a high grain diet. Lactic acid, in particular, is produced directly from starch fermentation, and, because it is not metabolized by the host animal, instead of being absorbed through the rumen wall, it causes an increase in lactic acid in the blood and a decrease in blood pH.

If the host's diet is changed too quickly, ruminal VFA accumulates, lowering rumen pH and increasing *S. bovis* and *Lactobacillus* spp., which has a negative impact on the rumen microbiota and the host animal (Chen et al. 2012). The high forage diets result in high production (60–70%) of acetic acid than the propionic acid (15–20%) and butyric acid (5–15%) of the overall VFAs. More grain or finely ground forages, on the other hand, can reduce acetic acid production by 40% while increasing propionic acid production by 40%. Changes in VFA production are normally linked to a decrease in milk fat test. However, the high-grain diet effects remain highly controversial on the epimural bacterial community. In heifers, a high-grain diet affects epimural bacterial diversity but not density (Chen et al. 2011). The high-grain diet feeding caused the rumen epithelium barrier dysfunction and inflammation (Liu et al. 2013). Despite the fact that high-grain feeding shifted the epimural bacterial population dramatically, it consistently promoted the abundance of epimural bacterial genera including *Butyrivibrio*, *Mogibacterium*, *Ruminococcus*, and *Succiniclasticum* (Petri et al. 2013; Liu et al. 2015). The diet has the most significant influence on the ruminal microbiota composition; further, comparative studies across host species are needed. Furthermore, a better understanding of diets and their changes could lead to the development of a new manipulation strategy for improving the health and performance of ruminant hosts.

5.8.3 Geographical Locations

In spite of host species and diet, the rumen-inhabiting microbial community in ruminant livestock species is also influenced by the geographical locations (Henderson et al. 2015). The global rumen census project discovered that the dominant microbial communities found in rumen samples from a variety of ruminant organisms are divided into three categories, bacteria, archaea, and protozoa, all of which are strikingly similar in all parts of the globe (Henderson et al. 2015). The negative correlation of abundant groups was noticed within bacteria, archaea, and protozoa, while the continuous replacement effects between dominant groups within each of these. A strong positive correlation was found within bacteria, archaea, and protozoa, which may be by either cooperation or sharing similar requirements in the rumen, but no such strong associations were found between them. The microbial community variation in different geographical locations is due to the availability of the major energy-yielding substrates (from transformation of the ingested feedstuffs) for their growth in the rumen. Therefore, the impacts of host species and diet on the rumen microbiome community are also accounted over the different geographical locations.

5.8.4 Probiotic Effect

Probiotics are the natural health additives used to boost ruminant animal health and efficiency by altering the rumen microbiome community structure and maintain homeostasis in host animal (Uyeno et al. 2015). They are administrated in a single or in consortium form to have the positive effects on the animal performance and growth rate. In domestic ruminants like cattle, sheep, and goats, oral probiotic administration has been shown to increase feed efficiency, weight gain, and feed intake, as well as milk quality and yield (Puniya et al. 2015). Even though the beneficial effects of probiotics are reported, some contradictory results with no such effect have been noticed due to variations in the composition, dose, and type (as microbial strains) of probiotics and the difference in the breeds of ruminants (Markowiak and Śliżewska 2018). The effects of different probiotics on the ruminants are discussed later in this chapter.

5.8.5 Effect of Antimicrobial Resistance

Antibiotic use in animals has increased in recent years, resulting in an increase in antimicrobial resistance in livestock farming systems. It endangers the welfare of both animals and humans. Antibiotics (also known as antibacterial) are drugs that are used to treat and prevent bacterial infections in humans and animals. They are not completely absorbed by the body but are extensively excreted via urine and feces. It has been reported that antibiotics can be introduced into the natural environments via soil fertilization with common use of various livestock manure as an organic fertilizer (Manyi-Loh et al. 2018). Furthermore, an antibiotic-polluted atmosphere may be a source of bacterial opportunistic pathogens that are already antibiotic-resistant or may acquire antibiotic resistance genes from other bacteria (Bengtsson-Palme et al. 2018).

Antimicrobial resistance has emerged as the most serious threat to human and animal health today. A better understanding of the antimicrobial resistance and pathogenicity genes' abundance in the animal gut provides the possible ways to reduce the problem and increase livestock production. The diversity and abundance of antimicrobial resistance and pathogenicity genes were higher in the concentrate-based diet-fed animals with increase in the relative abundance of *Proteobacteria* (which include many human and animal pathogens) (Auffret et al. 2017). Besides, these genes have the potential to be useful biomarkers for predicting animal health risk in the core ruminal microbiome.

5.8.6 Ruminal Disorders

A series of digestive and metabolic disorders such as ruminal and metabolic acidosis, rumenitis, bloating (rumen distension), liver abscess, rumen ulcer, laminitis, nitrate toxicity, and ammonia poisoning are considered as detrimental to the production and health of host ruminants (Russell and Rychlik 2001; Millen et al. 2016). The key practices required to prevent these problems are the adaptation of the rumen to high-grain diets and the reduction of readily fermentable carbohydrates. This necessitates a diet with a proper mix of fiber and non-fiber carbohydrates (starch) as well as excellent feedstuff bunk management. In ruminant animals, adding direct-fed microbial and ionophores to the diet can help reduce these risks.

5.9 Impact on Livestock Production

The rumen microbiome's symbiotic relationship with the ruminant host leads to the difference in many phenotypic traits of the host. The changes in either anyone of them will affect animal health and performance. Depending on the geographical regions, environmental conditions, and economic situation, each ruminant species can face different challenges during its existence. Ruminants have an exceptional ability to turn low-quality forages into high-quality end products such as milk and meat products. Thus, they are considered to serve a valuable role in the sustainable agricultural systems. For long-term livestock production, a variety of biotechnological approaches will be needed (Castillo et al. 2017). Additionally, studying the interaction of ruminal microbes with the physiological traits of ruminant hosts (such as composition and yield of milk and meat, efficiency of feed utilization, and methane emission) will be helpful for the improvement of these host traits by host's genetic modulation strategies as well as microbial manipulation tools (Malmuthuge and Guan 2017).

5.9.1 Milk and Meat Composition

Milk and meat are considered as the essence and integrity of the global livestock production systems which is the most important factor in rural empowerment. Consumption of milk and meat provides nutrition to human beings due to their nutritional characteristics (such as antioxidant and antimicrobial properties). The most active antioxidant in fat milk, conjugated linoleic acid (CLA, also known as rumenic acid or bovinic acid), is a unique component of ruminant's milk and meat with cytotoxic toward human mammary MCF-7 cancer cells (O'Shea et al. 2000). Natural *trans* isomers of fatty acids (such as CLA and vaccenic acid) are found in lipids produced by ruminants (like cattle, sheep, and goats) and account for about 4–6% of all fatty acids. The rumen bacteria (such as *Butyrivibrio fibrisolvens*, *Clostridium cellobioparum* and *lochheadi*) that synthesize isomerases and hydrolases transform *cis* fatty acids to *trans* fatty acids including linoleic acid, α-linolenic acid, oleic acid, and vaccenic acid (Grażyna et al. 2017). Rumen lipases hydrolyze the feed triglycerides and release *cis* configuration of free fatty acids which are later transformed by the rumen bacteria into their bioavailable forms. Rumen biohydrogenation and isomerization lead to separate production of *cis* and *trans* isomers of linoleic acid, α-linolenic acid, oleic acid, and vaccenic acid.

From more than 2000 milk-producing animal species, only a few of them have been domesticated for dairy production by humans in order to satisfy their own nutritional requirements. The various physiological processes in host animal are directly correlated with the abundance of specific rumen bacteria (*Bacteroidetes*, *Firmicutes*, and *Proteobacteria*). The ratio of *Firmicutes* to *Bacteroidetes* has been shown to be positively linked to daily milk fat yield (Li et al. 2009). Despite the fact that milk and dairy products are recommended in global dietary guidelines, lactose intolerance, milk protein allergy, and epidemiological studies back up their nutritional significance in the human diet, and sufficient milk and dairy product intake supports their beneficial function in the prevention of chronic diseases (such as cardiovascular diseases, metabolic syndrome, type 2 diabetes, and urinary bladder cancer) (Gil and Ortega 2019). In recent years, some functional products from ruminant animals have been produced due to the high demand of healthy foods with good taste by the growing human population. In order to minimize methane emissions, future research study on the ruminal microbiome may further help for the improvement of ruminal biohydrogenation that can adjust the milk and meat composition via the supplementation with the traditional medicinal plant extracts and natural products (Castillo et al. 2019).

5.9.2 Efficiency of Feed Utilization

The ever-increasing global human population growth is driven by the rapid increase in the demand for livestock's foods and feeds that tend to be derived from agricultural residues and by-products. Due to the largest impact of feed expenditure on long-term returns, the improvement in the efficiency of feed utilization is considered to be very important in order to lower the cost of livestock production. However, several feed efficiency traits [such as feed conversion ratio (FCR, or feed conversion rate), partial efficiency of growth (PEG, energetic efficiency for average daily gain), equilibrium maintenance efficiency, lactation efficiency, and residual feed intake (RFI)] have been proposed and used to increase the efficiency of animal production (Archer et al. 1999). Based on the RFI estimate, the rumen microbial community richness and diversity are closely related to divergent feed conversion efficiency trait in ruminants (sheep and cattle) (Hernandez-Sanabria et al. 2012; McLoughlin et al. 2020).

Significant differences in the microbial population shifts and operational taxonomic hierarchy with respect to efficiency of feed utilization have identified the dominant phyla (*Firmicutes* and *Lentisphaerae*), family (*Helicobacteraceae, Lachnospiraceae*, and *Veillonellaceae*), and genera (*Acidaminococcus*, *Anaerovibrio*, *Dialister*, *Janibacter*, *Lactobacillus*, *Leucobacter*, *Lysobacter*, *Prevotella*, *Succiniclasticum*, and *Ruminococcus*) in all ruminal groups (Myer et al. 2015). The specific bacterial phylotypes (such as *Eubacterium*, *Robinsoniella*, and *Succinivibrio* sp.) contributed to significant differences in feed efficiency traits that eventually impact the productivity of host which may be either dependent or independent from differing host diets (low-energy density diet and high-energy density diet) (Hernandez-Sanabria et al. 2012). The correlations detected by using culture-independent methods (such as PCR-DGGE and qRT-PCR analysis) were between dry matter intake, average daily gain, and 16S rRNA gene copy number of *Succinivibrio* sp. in efficient (low-RFI) steers, whereas the observed correlations between *Robinsoniella* sp. and RFI were for inefficient (high-RFI) steers. Since feed utilization efficiency is regarded as one of the most economically important characteristics, new techniques to boost this trait are in high demand. Thus, the rumen microbial species have the potential to use as the indicator traits for feed utilization efficiency as the variation in ruminant feed efficiency phenotypes and rumen microbial structure are related to each other with respect to feed efficiency.

5.9.3 Methane Production

The rumen's natural microbial fermentation and the hindgut involve the conversion of almost all plant polymers (which can't be broken down by the host's enzymes) to methane and CO_2 in an anaerobic environment. About 89% of methane is generated in the rumen and exhaled into the atmosphere by ruminants through their mouths and noses. During such substantial exhalation of methane, the ruminant animal suffers a loss of approximately 2–12% of dietary gross energy based on the dietary conditions (Hook et al. 2010). Methane, a highly flammable gas, is thought to be a viable potential energy source and a clean alternative to fossil fuels, as it can be used as a storable and transportable energy carrier, as vehicle fuel globally, and for the production of electricity and synthesis gas [syngas or water gas, i.e., a gas mixture of carbon monoxide (CO) and hydrogen (H_2)] (Ren et al. 2008).

Methane is a by-product of hydrogen production in the rumen by methanogenic archaea. Early in life, also in pre-ruminant stages, methanogenic microbes develop themselves (Guzman et al. 2015). Dietary improvements from a high-forage diet to a high-grain diet, methanogenic inhibitors (e.g., inhibitors of methanopterin biosynthesis, coenzyme M analogous, nitro compounds, and halogenated compounds), and supplementation of plant compounds are all possible mitigation strategies. These strategies primarily target methanogenic archaea by reducing substrate availability, either directly or indirectly (Tapio et al. 2017). However, because electrons aren't used in methane formation, inhibition of methanogenesis results in the accumulation of H_2. Methane mitigation strategies require the discovery of ruminal electron sinks that redirect excess H_2 to other pathways that are more favorable to host production (Ungerfeld 2013). The host genetic selection potential could be a viable option for ruminant methane emission reduction. Other heritable host physiological indicators (such as rumen size and retention time) that affect the ruminal microbiome may be potential indicator traits for indirect selection to reduce methane production. Moreover, the use of bacteriocin (such as nisin) and ionophore (such as monensin) has also been suggested as a possible way to reduce ruminal methane production, primarily by inhibiting the microbes required for the substrates formation (Shen et al. 2017).

5.10 Applications of Rumen Microbes

5.10.1 Biogas Production

The ruminal biomass contains a significant number of microbes that are capable of degrading nutrients through various metabolic processes and resulting in the production of biogas and biofertilizer, which in turn has a great potential for generating energy. The biogas represents a mixture of different gases (CH_4, CO_2, N_2, O_2, H_2, and H_2S) produced as a result of the conversion of volatile solids by the action of anaerobic microbes on domestic and agricultural wastes. Usually, it contains $\geq 50\%$ CH_4 with other gases in relatively low percentage. It is a renewable source of energy which is produced when organic matters [rumen content from ruminant animals (cattle, sheep, goats, etc.), cow dung, poultry droppings, pig excreta, human excreta, kitchen waste, etc.] and leftover agriculture products are easily fermented at low cost. Biogas production and its composition depend on the anaerobic digestion process' performance which is influenced by several factors such as organic loading rate, temperature, pH, pressure, carbon/nitrogen (C/N) ratio, retention time, etc. The rumen is not completely an anaerobic environment, and its biogas composition is different from biogas digesters and landfill sites (Table 5.1). The advantage is that it replaces other sources of energy such as wood-fuel (firewood and charcoal), electricity, liquefied petroleum gas (LPG), and crude oil, resolving environmental and agrochemical issues (Kelebe and Olorunnisola 2016). After animal excreta had been anaerobically fermented in the biogas production plant, the excreta become high-quality odorless fermented substrate which improves soil fertility with rich organic content better than fresh manure (Baştabak and Koçar 2020). As an energy source, it combats deforestation and pollution from organic wastes (i.e., kitchen waste, animal and human excreta) (Rasimphi and Tinarwo 2020).

Table 5.1 Composition of biogas produced in rumen fermentation, biogas digesters, and landfill sites

Gas	Rumen fermentation (%)	Anaerobic digesters (%)	Landfill sites (%)
Methane (CH_4)	22–29	40–80	54
Carbon dioxide (CO_2)	65–69	20–60	42
Nitrogen (N_2)	3–10	1–5	3.1
Oxygen (O_2)	0.5–1	Traces	0.8
Hydrogen (H_2)	0.18	0–3	–
Hydrogen sulfide (H_2S)	0.01–0.1	0.01–5	0.006

In addition to the environmental problems caused by conventional energy sources, there is another factor that requires careful attention of management of wastes generated from the slaughter of ruminant animals, which are often discarded into the environment without adequate treatment. This improper disposal of slaughterhouse/abattoir wastes leads to the intensification of environmental pollution due to the large amount of wastes. The disposal of ruminant abattoir waste poses a special challenge to the general public as it creates a nuisance to the environment as well as public eyesore. Therefore, use of these wastes for the generation of energy is a management strategy to the problem of waste disposal and pollution control (Franke-Whittle and Insam 2013; Audu et al. 2020).

Nowadays, the use of biogas is spreading from small farms to big commercial animal farms to enhance its energy security. Biogas can be directly used for cooking and heating by burning it in the presence of oxygen. It can also be converted into electricity in a steam turbine generator. Introducing biogas as a biofuel into existing vehicles can reduce global transport greenhouse gas emissions from 60–80% compared to fossil fuels (coal, petroleum, and natural gas) and liquid fuels (gasoline, diesel, and kerosene) (Scholwin et al. 2017). In the future, it is expected that biogas will be a valuable source of energy (Fig. 5.3) to preserve and protect the environment, solve the environmental pollution problem, and improve community health. After ruminant animals were slaughtered, the inoculation of the ruminal biomass has presented favorable conditions for the production of biogas and demonstrating the efficiency in the anaerobic digestion process (Chapleur et al. 2014). The fermentation features are compared between the rumen and typical anaerobic digestion processes (Table 5.2).

5.10.2 Industrial Applications of Rumen Microbial Enzymes

The rumen is an ideal microbial habitat conducive to survival and growth of microbes. Millions of microbes, including bacteria, archaea, protozoa, fungi, and viruses, live in the rumen reservoir and can produce a broad variety of enzymes. Hence, the rumen is considered a rich source of various enzymes which have the most prominent industrial and medicinal importance. Rumen microbial enzymes continuously play a vital role in the breakdown of lignocellulosic plant cell wall and non-structural carbohydrate (starch and sugars), nitrogenous compounds (proteins, amino acids, and urea), and lipids (Russell and Rychlik 2001). Like the production of VFAs from ruminal fermentation of carbohydrate, the microbial metabolism of nitrogenous compounds also attributes a key component of the microbial energy economy, since it promotes ruminal microbial protein synthesis, which is a source of amino acids for muscle protein accretion (Bach et al. 2005).

Rumen functioning is often relatively similar to multi-product biorefineries which are intended for bioconversion of lignocellulosic materials into various high-value bioproducts (such as biofuels, biochemicals, etc.). Several anaerobic ruminal fungi (*Anaeromyces* sp., *Orpinomyces* sp., *Piromyces* sp., and *Neocallimastix* sp.) are

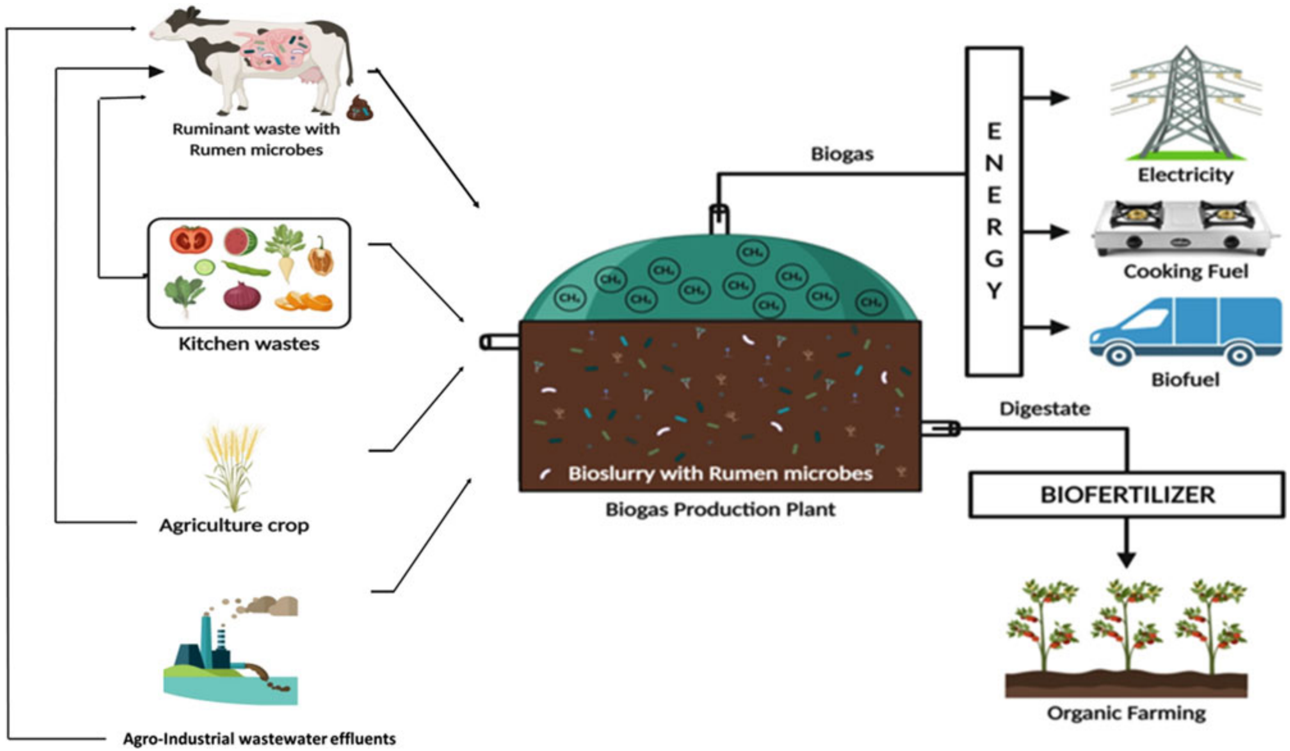

Fig. 5.3 Biogas production from cattle (*Bos taurus*) rumen biomass with microbes and its use as a source of energy

Table 5.2 Comparison of features of fermentation in rumen and anaerobic digestion

Characteristics	Rumen (Dehority 2002)	Anaerobic digesters (Sattler 2011)
Temperature (°C)	39	15–55
pH	Vicinity of 6.5 (in general); 5.5–7.0 (fairly well buffered)	6.8–7.5
Osmotic pressure (mOsm/kg)	250–400 (hypotonic to plasma)	–
Organic dry matter content (%)	10–13	0.5–40
Redox potential (mV)	Anaerobic with a range of -150 to -350	<-300
Solid retention time (SRT, days)	0.83–1.25	10–15
Hydraulic retention time (HRT, days)	0.25–0.66	20 (sheep manure); 28–38 (cattle manure)
Solids turnover time (days)	0.8–2.2 (sheep); 1.3–3.7 (cattle)	2–60
Volatile solids degradation (%)	40–75	21.5 (sheep); 7.98 (dairy cattle)
Threshold size of particles (mm)	1–2 (sheep); 2–5 (cattle)	1–3
Atmosphere (Principal gases)	CH_4 (27%) and CO_2 (65%)	CH_4 (60–70%) and CO_2 (30–40%)

actively involved in plant fiber degradation while creating a broad variety of potential hydrolytic and fibrolytic enzymes in the rumen (Thareja et al. 2006; Tripathi et al. 2007a; Paul et al. 2010). Apparently, Chen et al. (2014) have reported that a rumen fungal strain (*Orpinomyces* sp. Y102) isolated from cow with exhibiting β-glucanase activities was found to be a good candidate for industrial applications especially for the production of biofuels. The properties and industrial applications of rumen microbial enzymes are listed in Table 5.3.

5.10.3 Rumen-Based Products

Ruminal microbes play a critical role in maximizing feed nutrient use. Feedstuffs and microbial feed additives that are commonly incorporated into ruminant diets are known to alter the rumen microbial ecology and diversity. It is important to examine management techniques to enhance not only the gut microbiota's ability to absorb wasted nutrients from food waste sources but also the balance and activities of the rumen microbial flora when probiotics and prebiotics are administered (Parfitt et al. 2010; Uyeno et al. 2015). A number of microbes including bacteria, live yeast, and/or fungi (Fig. 5.4) are directly supplemented as probiotics to the ruminants

Table 5.3 Properties and industrial applications of rumen microbial enzymes

Enzymes	EC Number	Applications
Endo-β-1,4-glucanase	EC 3.2.1.4	Hydrolysis of cellulosic materials to sugars in biofuel manufacturing industry (Wilson 2009) Increase rate of wort filtration, reduce precipitation of β-glucan in beer, and reduce mash viscosity and turbidity in brewing and beverage industries (Celestino et al. 2006) Modify fiber and bioremediation of pulp and paper mill effluent in pulp and paper industries (Pandey et al. 2014) Cotton softening and denim finishing in textile industry (Sahin et al. 2016) As an additive for fabric softening and color brightening and removal of particulate soil components in detergent industry (Pandey et al. 2014) Degradation of β-glucan, increase feed digestibility, and overcome the antinutritive effect of (1,3;1,4)-β-D-glucan in poultry and animal feed industries (Mathlouthi et al. 2002)
Exo-β-1,4-glucanase	EC 3.2.1.91	Hydrolysis of non-reducing ends of cellulose chains to release cellobiose units in industrial cellulose degradation process (Baramee et al. 2017) Mechanical pulping in pulp and paper industries (Viikari et al. 2010) Biofinishing of cotton fabric in textile industry (Arja 2007) Fabric softening, improve color brightness, and removal of particular soils in detergent industry (Aehle 2007)
Endo-β-1,4-xylanase	EC 3.2.1.8	Hydrolysis of plant xylan for biofuel production in biofuel manufacturing industry (Gómez et al. 2016) Clarifying fruit juices in beverage industry (Adiguzel et al. 2019) Bread production and improve gluten strength, dough stability and flexibility in baking industry (McPhillips et al. 2014) Biobleaching of wood pulps to reduce use of chlorine and chlorine dioxide in pulp and paper industries (Walia et al. 2017) Enzymatic retting of flax, jute, ramie, and hemp in textile industry (Collins et al. 2005) Hydrolysis of β-glucan and arabinoxylan, improve feed conversion ratio and nutrient utilization, body weight gain, and animal production in animal feed industry (Dorn et al. 2018)
1,4-β-D-Xylosidase	EC 3.2.1.37	Used for improving baking properties of bread dough, improving digestibility of animal feed, production of D-xylose for xylitol manufacture, and deinking of recycled paper (Jordan and Wagschal 2010)

(continued)

Table 5.3 (continued)

Enzymes	EC Number	Applications
α-L-Arabinofuranosidase	EC 3.2.1.55	Used for improving bread quality, wine flavor, and clarification of fruit juices and as supplement for feedstock for enhancing digestion (Thakur et al. 2019)
α-Glucuronidase	EC 3.2.1.139	Production of chemical feed stocks and fuels and improve products in pulp and paper industry (Monclaro and Ferreira Filho 2014)
Ferulic acid esterase (feruloyl esterase)	EC 3.1.1.73	Saccharification in biorefinery industry (Underlin et al. 2020) Biosynthesis of ferulic acid (a vanillin precursor) and related fine chemicals from plant cell walls in food, beverage, perfumery, and pharmaceutical industries (Gallage and Møller 2015) Bio-bleaching in pulp and paper industries (Ozer et al. 2020) Improve the performance of liquid detergent in detergent industry (Liu et al. 2016) Improve fiber digestibility to make the bioavailability of phytonutrients, accelerate animal growth rate, and reduce immunological stress in animal feed industry (Lynch et al. 2014)
p-Coumaroyl esterase	EC 3.1.1.B10	Release of hydroxycinnamic acids (caffeic acid, ferulic acid, and *p*-coumaric acid) from the complex natural substrates (i.e., agro-industrial byproducts) (Nieter et al. 2017)
β-1,3-Glucanase	EC 3.2.1.39	Applied for degradation of glycans in food industry and to prevent spoilage in wine making process (Blättel et al. 2011)
Endo-1,3(4)-β-glucanase	EC 3.2.1.6	Animal (poultry) feed industry (Boyce and Walsh 2007)
Pectate lyase	EC 4.2.2.2 & EC 4.2.2.9	Food processing, textile industry, oil extraction, pulp processing, and industrial wastewater treatment (Wu et al. 2020)
Pectin lyase	EC 4.2.2.10	Used for juice extraction and clarification in beverage industry (Wu et al. 2020)
Pectin methylesterase	EC 3.1.1.11	Used in food, cosmetic, and pharmaceutical industries and to increase fruit products hardness and reduce juice turbidity in fruit juice industry (Jiang et al. 2013)
α-Amylase	EC 3.2.1.1	Anti-staling effect for improving bread quality in baking industry (Ait Kaki El-Hadef El-Okki et al. 2017) Brewing, starch liquefaction, and clarification of beer and fruit juices in beverage industry (Raveendran et al. 2018) Desizing of cotton fabrics in textile industry (Mojsov 2019) Enhance detergents ability to remove tough stains

(continued)

Table 5.3 (continued)

Enzymes	EC Number	Applications
		and making detergent eco-friendly in detergent industry (Hmidet et al. 2009) Corn starch hydrolysis for fructose and glucose syrups production in starch processing industry (Nielsen and Borchert 2000) Degradation of residues of starchy foods and removal of starch from the surface of fabrics in detergent industry (Roy et al. 2012) Increase degradation of starch in animal feed industry (Nozière et al. 2014) Rapid reduction of viscosity of gelatinized starch in oil drilling in petroleum industry (Kyaw et al. 2010)
β-Amylase	EC 3.2.1.2	Baking industry, brewing in beer and distilled alcohol production in beverage industry, and starch processing for glucose syrups production (EFSA CEF Panel 2017)
Glucoamylase	EC 3.2.1.3	Brewing of low-calorie beer (light beer), hydrolysis of starch and whole grains for alcohol production in beverage industry (Raveendran et al. 2018) Hydrolysis of starch for production of high-glucose and high-fructose corn syrups in starch processing industry (Raveendran et al. 2018) Improve flour, dough, and bread quality and retard dough staling in baking industry (Raveendran et al. 2018)
Proteases	–	Brewing, meat tenderization, coagulation of milk, and bread quality improvement (Raveendran et al. 2018)
Protein tyrosine phosphatase (a new class of phytase)	EC 3.1.3.48	Utilization of phytate phosphorus, release of phosphate, and reduce environmental pollution caused by excess phosphorus in animal diets in animal feed industry (Nakashima et al. 2007) Release of minerals (Fe, Ca, Zn, and Mg) in cereals to enhance their bioaccessibility in food industry (Sharma et al. 2018)
Tannase (tannin acyl hydrolase)	EC 3.1.1.20	Enzymatic clarification of beer and fruit juices rich in tannins, treatment of malt polyphenols for beer stabilization, improvement in flavor of wines, and manufacturing of coffee flavored soft drinks and instant tea in beverage industry (Govindarajan et al. 2016) Food detannification and synthesis of food preservatives (such as pyrogallol and gallates) in food industry (Belmares et al. 2004) High-grade leather tannins preparation and bioremediation of toxic tannery effluents in leather industry (Dhiman et al. 2017; Cavalcanti et al. 2018)

(continued)

Table 5.3 (continued)

Enzymes	EC Number	Applications
		Mass production of gallic acid from tannic acid for synthesis of antioxidant propyl gallate and antibiotic trimethoprim in food and pharmaceutical industries (Aguilar-Zárate et al. 2015) Treatment of green tea and its polyphenolic components to inhibit carcinogenic, mutagenic, and teratogenic effects of *N*-nitrosamines (Lu and Chen 2007) Inhibit sediment formation and improve color stability, taste quality, and organoleptic properties in green tea (Wang et al. 2020) Degradation of anti-nutritional hydrolysable tannins (HTs) and euptox A to show potential role as microbial feed additives to increase plant biomass utilization and improve animal digestibility in animal feed industry (Sharma et al. 2017)

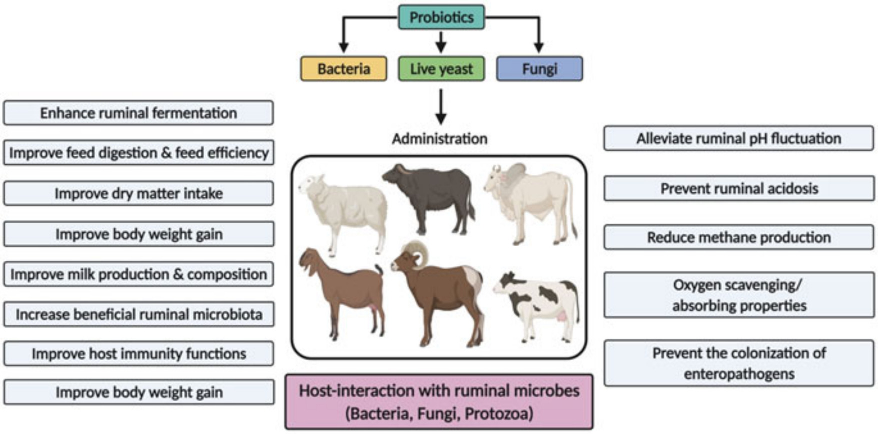

Fig. 5.4 Host-interaction with rumen microbes after administered with the probiotics and prebiotics

solely through their diet which help establish the healthy microflora in the rumen and gut by microbial imbalance (dysbiosis) and undesired pH changes. Mainly, they have been administered to enhance ruminal fermentation and fiber digestion, manipulate the beneficial ruminal microbiota, improve host immunity functions, and prevent the colonization of enteropathogens.

5.10.3.1 Bacterial Supplements

Bacterial probiotics can help ruminants improve their dry matter intake, feed utilization efficiency, body weight gain, and milk yield by improving rumen conditions (Elghandour et al. 2015). They also inhibit the growth of pathogens and modulate the host immune function through the production of potent antimicrobial compounds (such as VFAs, hydrogen peroxide, nitric oxide, and bacteriocins) as well as improving rumen and gastrointestinal tract microbial balance (Raabis et al. 2019). Despite the fact that many bacteria from the rumen and other intestinal environments have been isolated, characterized, and established, small numbers of bacteria are reintroduced into the rumen, along with their residual persistence. A bacterium found in the rumen *Synergistes jonesii* degrades the toxic compound 3-hydroxy-4 (1H)-pyridone (3,4-DHP), which is formed in the rumen from mimosine (a non-protein amino acid that causes a problem in ruminants due to *Leucaena* feeding) contained in the leaves and seeds of *Leucaena leucocephala* (Allison et al. 1992). Lactate-producing bacteria (*Bacillus*, *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, and *Streptococcus*) that sustain lactic acid (or lactate) are a potential means of reducing rumen acidosis in animals fed a high-concentrate diet (Lettat et al. 2012; Uyeno et al. 2015). Besides, as stimulants of major lactate utilizers, the administration of *Megasphaera elsdenii*, *Prevotella bryantii*, *Propionibacterium shermanii*, *Streptococcus bovis*, and *Selenomonas ruminantium* has been reported as alleviating fermentation and preventing acidosis in rumen (Weimer 2001; Long et al. 2014).

5.10.3.2 Live Yeast Cell Supplements

Furthermore, live yeast cells have the ability to serve as both a probiotic and a prebiotic in the rumen. Probiotic yeast supplementation is used to treat rumen microbial dysbiosis, which increases nutrient utilization; enhances ruminant productivity, milk production, and immunity; reduces the number of infectious pathogens; and protects the livestock's global environment. The addition of probiotic yeasts modulates the composition and activities of rumen microbial ecosystem and enhances the microbial balance in ruminant stomach by producing both intracellular and extracellular metabolites. This also leads to a decrease in lactic acid content by competing with lactate-producing bacteria for substrate, alleviates ruminal pH fluctuation, improves nutrient digestibility, optimizes the proportion of VFAs, decreases the ammonia nitrogen (NH_3-N) production, and stimulates microbial population in rumen (Chaucheyras-Durand et al. 2012; Wang et al. 2016). Alteration of ruminal fermentation to reduce the production of methane and consequent decrease in the energy losses during fermentation is another positive effect of dietary yeasts (Ruiz et al. 2016). Despite the fact that almost all yeasts are obligate aerobic microbes, they have oxygen-scavenging/oxygen-absorbing properties in order to maintain the anaerobiosis needed for optimal fermentation (Chaucheyras-Durand et al. 2012).

Nutritional yeasts used as supplements in animal feeds elicit the prebiotic effect through some nutraceutical compounds (proteins, peptides, amino acids, lipids, vitamins, and organic acids) present within yeast cells and cell walls (β-glucans, mannan-oligosaccharides, and nucleotides) which can stimulate the rumen microbial communities with improving ruminant growth and health (Alugongo et al. 2017; Shurson 2018). Therefore, the dietary supplementation of live yeast cells can have a positive effect consequently on ruminal efficiency.

Baker's yeast (*Saccharomyces cerevisiae*) is the most frequently used yeast strain in ruminant feeding, and it can help stabilize ruminal pH, thereby activating the fiber-degrading bacterial population in the rumen and enhancing plant fiber digestion. Pinloche et al. (2013) have reported that the relative abundance of lactate-utilizing bacteria (such as *Megasphaera* and *Selenomonas*) was stimulated with diet supplementation of probiotic live yeast as well as the starch-utilizing bacteria and fibrolytic bacteria (such as *Fibrobacter* and *Ruminococcus*) within the rumen of cattle, and thus, the results confirm the mode of action of yeast in declining lactic acid accumulation and helping ruminal pH regulation. The yeast supplemented diets have positive effects in increasing dry matter digestion and VFAs production, and decreasing tendency in acetate to propionate ratio, formation of methane and ammonia gas, thus subsequently increase in milk production in dairy cows (Chung et al. 2011; Uyeno et al. 2017).

Other yeast species (*Candida utilis* and *C. tropicalis*) were used as supplements in addition to *S. cerevisiae* to increase fermentation synergistically, as evidenced by maximum gas output, absence of dry matter and neutral detergent fiber, and lower methane production (Wang et al. 2016; Kong et al. 2019). Intensive system (high input-high output) of animal production needs to stimulate fast muscle growth and higher milk production by feeding a high-concentrate diet that induces rumen microbial dysbiosis and metabolic diseases and disorders [lameness, laminitis, mastitis, subacute ruminal acidosis (SARA), etc.]. These lead to increase of a host of health problems and environmental risks. These perturb the normal rumen functions eventually leading to reduced feed intake and poor animal health and productivity. Thus, supplementing yeast culture to the ruminant diets can be a common practice in order to maintain the normal rumen functions and overall health of animals.

5.10.3.3 Fungal Supplements

Generally, the anaerobic rumen fungi (*Aspergillus oryzae*, *Neocallimastix* sp., *Orpinomyces* sp., *Piromyces* sp.) can be administered as direct-fed microbials to increase nutrient digestibility, rumen fermentation, milk production, and growth rate (body weight gain) in ruminants (Tripathi et al. 2007b; Sehgal et al. 2008; Saxena et al. 2010; Puniya et al. 2015). Rumen fungi administered reported to increase the rumen NH_3-N during fermentation which is further used in proliferation of bacteria and microbial protein synthesis. The ruminants administered with anaerobic fungi also significantly increased the digestibility of dry matter intake, crude protein, neutral detergent fiber, and acid detergent fiber (Sehgal et al. 2008; Saxena et al. 2010). In addition to improvement in the digestibility of diets, the direct-fed fungi also increase the total rumen anaerobic fungi and cellulolytic bacteria (Puniya et al. 2015). Lee et al. (2000) have reported that the direct administration of culture of an anaerobic fungal strain, *Orpinomyces* strain KNGF-2, to the rumen of sheep enhanced the digestibility of nutrients and nitrogen retention by increasing the number of rumen microbes (bacteria and fungi) and altering the production pattern of VFAs. However, the administration of fungal enzymes to the rumen failed to improve ruminal fermentation, digestibility of nutrients, and nitrogen retention due to rapid degradation of proteins by ruminal microbes and their enzymes. Paul et al. (2010) have suggested that fibrolytic fungi could be administered as microbial additives into domestic ruminants for the improvement in utilization of nutrients from poor-quality lignocellulosic feeds. There are limited studies on the quantification of persistence and successful colonization of introduced fungi in the rumen.

5.10.3.4 Plant Secondary Metabolites

Plant secondary metabolites (flavonoids, glucosinolates, mimosine, saponins, tannins, essential oils, etc.) can affect a number of physiological and metabolic processes which alter the microbial activity, reduce digestion, and compromise the process of maintaining acid-base homeostasis and toxicity/ poisoning (Estell 2010). They're also known as phytonutrients or phytobiotics, and they're used in ruminant animal feeding as an alternative to rumen modifiers (also known as ionophoric antibiotics) (Oh and Hristov 2016). These metabolites function as pathogen inhibitors (bacteria, fungi, and yeast), improving animal immunity, health, and productivity. Secondary metabolites' negative and positive effects are defined by their biosynthetic origin, chemical structure, and dietary concentration.

The ruminant animals supplemented with tannins (hydrolysable and condensed) result in inhibition of the growth and activities of rumen proteolytic bacteria (such as *Butyrivibrio fibrisolvens*, *Clostridium proteoclasticum*, *Eubacterium* sp., *Ruminobacter amylophilus*, and *Streptococcus bovis*), thereby preventing the bacterial proteolysis in rumen by forming the insoluble tannin-protein complexes (Min et al. 2002; Patra and Saxena 2011). The dietary tannins could contribute to reduce the methane production by decreasing the size of population and diversity of rumen bacteria, methanogenic archaea, and protozoa (Saminathan et al. 2016; Yang et al. 2017). Furthermore, dietary tannins prevent frothy bloat, a severe digestive disorder in ruminants that occurs when gas output exceeds the animal's capacity to remove gas through eructation or when the eructation mechanism is inhibited (Patra and Saxena 2011; Wang et al. 2012). Therefore, recent findings in ruminant nutrition researches recommended the supplementation of trace amounts of secondary metabolites into ruminant diets to improve the feed utilization, manipulate rumen fermentation, and reduce methane production (Ku-Vera et al. 2020).

5.10.3.5 Ionophores

Ionophores are antimicrobial compounds that are widely fed to ruminant animals to improve feed effectiveness, body weight gain, and coccidiosis control (Callaway et al. 2003). Globally, there are seven ionophores (including laidlomycin, lasalocid, maduramicin, monensin, narasin, salinomycin and semduramicin) approved for prophylactic use as anticoccidial (coccidiostat) drugs as well as growth promoters in ruminants. They improve animal productivity and health by altering the composition of rumen microbial communities, thereby attenuating the incidence and severity of certain ruminal digestive disorders (such as acidosis, frothy bloat, and acute bovine pulmonary edema and emphysema) (McGuffey et al. 2001; Tedeschi et al. 2003). However, due to their complexity and high degree of specificity of resistance mechanisms, ionophores, like all other antimicrobial compounds, do not contribute to the production or spread of antibiotic resistance. As a result, ionophores will continue to be an effective tool in improving the productivity of animal production in the future.

5.11 Future Prospective and Conclusion

Future advanced technologies will most likely fill the current technology gap by representing microbial communities and enhancing our understanding of how they function cooperatively using assembled mock communities and systems biology approaches. For decades, biotechnologists, microbiologists, and nutritionists have studied the rumen microbiome with the aim of improving ruminant efficiency by altering dietary components or modifying microbial ecosystems. For a better understanding of microbial sequence signature, genomics methods are used to discover novel microbes or genes. The isolation and commercialization of most widely used microbial enzymes from the rumen, such as xylanase, endoglucanase, and esterases, must accompany in a new age for agro-industries. The use of genetic engineering to resolve numerous problems by adding desirable activities within the formed rumen microbes is a solution, but some problems remain unsolved, such as the rumen microbes' shelf life.

In silico tools are used to predict inhibitors against the active sites of various enzymes and to regulate rumen methane production, so these tools will help reduce the amount of energy, manpower, and time spent on research. For example, the bioinformatics tool predicted that lovastatin and compactin (mevastatin) are potential inhibitors of *Methanobrevibacter smithii*'s F420-dependent NADP oxidoreductase protein, which catalyzes the crucial electron transfer step during methanogenesis (Sharma et al. 2011). Chemical inhibitors for the methanogenesis process have been identified using in vitro and in silico approaches. Ruminal methanogens are rarely isolated into pure cultures from ruminants (Patra et al. 2017). Based on the global data of rumen microbiome, *Methanobrevibacter* is considered the dominant rumen methanogen microbe among the genome sequences (Matthews et al. 2019). Genome sequencing projects have provided information on rumen microbes and their metabolites, as well as their effect on ruminant hosts' immune responses and gastrointestinal activities. They also provide information on the host's biology and interactions with resident rumen microbes in relation to ruminant health and nutrition, which can be used to produce prebiotics and probiotics.

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Chapter 6
The Potential of Cow's Dung in Sustainable Agriculture and Environmental Health

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Abstract Cow worship in India has a long history dating back to the Vedic era, several thousand years ago. Cow being a domestic animal, its milk is as nourishing as one's mother's milk, and its utility in a primarily agricultural civilization like India made it a highly beneficial animal whose dung and urine played a key role in Indian medicine system as well as ancient India's religious rituals. *Ayurveda* and *Charaka Samhita* are the examples of Indian writings having detailed description of immense medical, health and general wellbeing benefits of various products obtained from cow. Cow products such as milk, yoghurt, refined butter ghee and even cow dung were regarded as highly useful medicines in ancient India's medicine system and till date continue to be utilized in Indian household as medicines of great value. Cow products are examined in modern science as well and have been discovered to be quite effective in the treatment and prevention of numerous ailments. Studies on deep microbial diversity of cow dung have been carried out using modern scientific equipment, and these studies provide a coherent insight into the microorganism's synergistic potential. Compost (dung manure) is widely used in Indian agriculture, indicating its potential for increasing crop output. Considering many uses of cow dung, the scientific community is becoming increasingly interested in its scientific analysis and use, especially now that organic farming and indigenous medicines are garnering a lot of attention across the world.

Keywords Cow dung · Panchgavya · Cow urine · Biofertilizer · Antibacterial · Antifungal materials

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_6

6.1 Introduction

The Vedic civilization of India extends back to several thousand years, and it has spread throughout the world as a result of the Indus Valley's influence on people's lives and cultures. The learned Rishis, Seers and Sages of Vedic India have left behind a treasure trove of knowledge in the form of the Vedic texts, which were compiled as the four Vedas by Maharishi Vedavyasa during the Mahabharata period some 5000 years ago and are still the most ancient texts composed in a natural scientific language, Sanskrit, which is also the mother tongue of many Asian and European languages. These Vedic Texts are classified as Rig Veda, Yajur Veda, Sam Veda and Arthur Veda along with the associated philosophical treatises called Upanishads containing rich knowledge of creation and ordaining a lifestyle based on the principles of righteousness, truthfulness, peace and harmony with nature. The Vedas also provide emphasis on integration of science and spirituality to create such a righteous society that protects the interest of man as well as Mother Nature. The Vedas acknowledge "Cow" as a sacred "holy mother", a saviour of life and an embodiment of all gods and goddesses that bestow the bliss of havens on earth. The cow breed Kamdhenu has been referred by the Vedic Seers as the most sacred Cow as per the Gou-mantra given below.

सर्वकामदुधे देवि सर्वतीर्थीभिषेचिनि ।।
पावने सुरभि श्रेष्ठे देवि तुभ्यं नमोस्तुते ।।

Sarvakaamdudhe Devi Sarvatithirbhishechini l
Pavane Surbhi Shreshte Devi Tubhyam Namostute ll

Meaning thereby that Kamadhenu is revered as a celestial wish-fulfilling cow. All Gods, Goddesses and the Holy Trinity of the gods of creation, sustenance and destruction all dwell in the holy cow. Those who worship cows are rewarded with good fortune. Further, according to Vedic literature, a cow's "Suryaketu" nerve absorbs harmful radiation and cleanses the environment. In Ayurveda and Charaka Samhita, Panchgavya (a formulation based on five cow-derived products) is considered a medicine and plays a key role in disease prevention and management. In India, cow dung is utilized as an environmental preservative, disinfectant, source of humic acid elements to improve organic fertilizer quality and pest repellent in rural households. Plants containing cellulose, hemicellulose and lignins provide the animal's sustenance. Organic matter, including fibrous material, passes through the cow's digestive system as liquid processed following fermentation, absorption and filtering, and is not fully *digested* but rather annihilated by the rumen's microbial community. The most important chemical factors are carbon, nitrogen, hydrogen, oxygen, phosphorus, salts, urea and lignocellulose (Rajeswari et al. 2016). When compared to cross-breed cow manure, native cow dung has higher quantities of calcium, phosphate, zinc and copper (Randhawa and Kullar 2011). Because of its

Table 6.1 Physio-chemical characteristics of cow dung (Source: Bhatt and Maheshwari 2019)

S. no.	Parameters	Value
1	pH	7.1–7.3
2	Electric conductivity (ds/m)	0.97
3	Organic carbon (%)	26.27
4	Nitrogen (%)	0.924
5	Phosphorus (%)	0.274
6	Potassium (%)	1.025

diverse microbiological life, cow dung is classified as organic manure (Table 6.1). The Indian government has undertaken several programmes, including the "National Programme for Bovine Breeding and Dairy Development", the "National Dairy Plan (Phase-I)", and the "Dairy Development Program" and the "Dairy Entrepreneurship Development Scheme", as well as the "Rashtriya Gokul Mission", to protect cows and strengthen the dairy sector. Haryana's cow protection law, Haryana Gauvansh Sanrakshan and Gausamvardhan (Amendment) Act, 2019, prohibits cow slaughter, trafficking and sale of beef in the state, recognizing the importance of this objective (Scroll 2019). While asserting scientific benefits from cow protection and investigating possible uses of cow products, the Gau Vigyan Anusandhan Kendra (Cow Science Research Centre) in Nagpur was established to do research on "cow science" (The Hindu 2015). Cows are the most sacred and beneficial creatures in the universe, according to the "Gavopanishad" (taught by Maharshi Vasishta), as shown below.

घृतनद्यो घृतावर्तास्ता मे सन्तु सदा गृहे॥

घृतं मे हृदये नित्यं घृतं नाभ्यां प्रतिष्ठितम्।

घृतं सर्वेषु गात्रेषु घृतं मे मनसि स्थितम्॥

गावो ममाग्रतो नित्यं गावः पृष्ठत एव च।

गावो मे सर्वतश्चैव गवां मध्ये वसाम्यहम्॥

Ghr̥itanadyo ghr̥itavartasta me santu sada gr̥he. Ghr̥tam me hr̥idaye nityam ghr̥atam
nabhayam pratiṣṭhitam. Ghr̥ataṁ sarveṣu gatreṣu ghr̥itam me manasi sthitam. Gavo
mamagrato nityam gavaḥ pr̥aṣṭhata eva ca. Gavo me sarvatascaiva gavam madhye vasham

This Sanskrit scripture emphasizes the importance of refined cow "ghee", stating that "the source of "ghee", the river of ghee and the whirlpool form ghee cows should always live in my house. "Cow's ghee" has always been in my heart. Ghee should be distinguished in my navel. Let the ghee pervade my entire limbs, and let the "ghee" be located in my mind. Let the cows be around me, and let me dwell among the cows. It's little wonder that the cow continues to be one of the most respected household animals in India's spiritual civilization. Cows are responsible for the world’s ability to survive. Cows are auspicious, sacred and bringer of good luck. Cows provide people with milk, yoghurt, cheese, butter and “ghee”, among other things. Cow’s milk is like ambrosial nectar, according to Vedanta, and ghee prepared from cow’s milk is the best of all the food products in the household. Advances in cow science have made it easier to analyse scientific evidence of potential applications of directly and indirectly generated cow products that contribute to long-term environmental health. As we return to our roots and work to focus on sustainable developments, the importance of cows in agriculture economies for promotion of organic agriculture and green agri-technologies would require much greater attention to both cow dung and its urine for their natural fertilizer properties and restoration of the ecosystem for a sustainable future for the mankind. In this chapter, we focus on cow dung and its characteristics and application in the modern context (Table 6.2).

6.1.1 Vedic History of Cows in Indian Culture and Legacy

The supreme personality Maharishi Vaisyas (18.44) has advised in the Bhagavad Gita that “farming, cow protection, and trade are regarded nature work”. As a result, farmers have a natural responsibility to protect cows.

Charaka Samhita states, “milk is considered as the best life strengthening”. Milk contains casein protein, which aids newborn growth, calcium and sulphur, which strengthen bones, and Vitamin D and B-complex vitamins. Curd helps avoid diarrhoea, enhance fat digestion and fight cancer. “Ghee” is a brain and beauty enhancer that also helps keep eye disorders at bay. The disease-fighting qualities of distilled cow urine are numerous.

Panchagavya Mix: Many Ayurvedic formulations, such as Panchagavya Ghrita, Amritasara, Ghanavati, Ksharvati and Netrasara, are essential medications. Panchagavya can be taken alone or in conjunction with Ayurvedic drugs. The Panchagavya ingredients have been scientifically analyzed. It’s been shown to help people recover from a number of ailments and boost their immunity to a variety of diseases. Cow dung possesses antifungal, antibacterial, and suppressive properties against pathogenic agents, making it as effective as pharmaceutical formulations in crop disease management. This property of cow dung makes it a natural cleanser and an environmentally friendly organic alternative to inorganic toxins. Cow dung contains humic acid as well as a variety of important elements, making it a possible source of protein for microbes. Due to its high microbial load, cow dung is the best and fastest decomposer of organic compounds. When it comes to regaining the status of agricultural soil, organic matter is a critical component.

Table 6.2 Microbial diversity of cow dung

Class	Microorganism	Reference
Bacteria	*Stenotrophomonas*, *Achromobacter*, *Pseudomonas* and *Brevibacillus*	Aiysha and Latif (2019)
	Bacillus, *Bifidobacterium*, *Lactobacillus*	Teo and Teoh (2011)
	Paenibacillus flaviporus	Velazquez et al. (2004)
	Bacillus pumilus, *Bacillus sphaericus*, *Bacillus macerans*, *Bacillus laterosporus*, *Micrococcus varians*, *Proteus mirabilis* and *Enterobacter aerogenes*	Adegunloye et al. (2007)
	Citrobacter koseri, *E. aerogenes*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Kluyvera* sp., *Morganella morganii*, *Pasteurella* spp., *Providencia stuartii* and *Pseudomonas* spp.	Sawant et al. (2007)
	Acinetobacter spp., *Bacillus* sp., *Serratia* sp., *Alcaligenes* sp. and *Pseudomonas* sp.	Akinde and Obire (2008)
	Bacillus safensis (PG1), *Bacillus cereus* (PG2, PG4, PG5), *Bacillus subtilis* (BD2), *Lysinibacillus xylanilyticus* (BD3), *Bacillus licheniformis* (CPP1)	Radha and Rao (2014)
	Proteobacteria	Lv et al. (2015)
Actinomycetes	*Nocardia* spp.	Radha and Rao (2014)
	Rhodococcus coprophilus	Rowbotham and Cross (1977)
	Micromonospora chalcea, *Pseudonocardia thermophila*	Godden et al. (1983)
	Streptomyces spp.	Semwal et al. (2018)
Fungi and yeasts	*Neocallimastix frontalis* *Piromyces communis* *Orpinomyces joyonii*	Moniello et al. (1996) Dashtban et al. (2009) Hodrova et al. (1995)
	Aspergillus niger, *Aspergillus flavus*, *Aspergillus repens*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Mucor mucedo*, *Fusarium* spp. and *Vericosporium* spp.	Adegunloye et al. (2007)
	Alternaria sp., *Aspergillus* sp., *Cephalosporium* sp., *Cladosporium* sp., *Geotrichum* sp., *Monilia* sp., *Mucor* sp., *Penicillium* sp., *Rhizopus* sp., *Sporotrichum* sp., *Thamnidium* sp.	Adegunloye et al. (2007)
	Candida sp., *Rhodotorula* sp., *Saccharomyces*, *Sporobolomyces*, *Trichosporon* and *Torulopsis* sp.	Okwute and Ijah (2014)
	Blastomyces sp., *Botryodiplodia theobromae*, *Fusarium* sp., *Nigrospora* sp., *Penicillium chrysogenum*, *Penicillium glabrum*, *Pleurofragmium* sp. and *Trichoderma harzianum*	Okwute and Ijah (2014)

6.2 Characteristics of Cow Dung

Cow dung is a 3:1 mixture of faeces, cellulose and hemicellulose produced by herbivorous bovine animals as an undigested remnant of consumed food (Gupta et al. 2016). Among these are "nitrogen (N), phosphorus (P), potassium (K), iron (Fe), sulphur (S), magnesium (Mg), calcium (Ca), cobalt (Co), manganese (Mn), and chlorine (Cl)" (Garg and Mudgal 2007).

6.3 Microbial Diversity of Cow Dung

Bacilli, lactobacilli and cocci, as well as fungi like *Pilobus* and yeasts both recognized and unknown, are abundant in the microflora of cow dung (Rajeswari et al. 2016). Nearly 60 bacterial species, the bulk of which are "*Bacillus* sp., *Corynebacterium* sp., *Lactobacillus* sp. and a few fungal species such as *Aspergillus* and *Trichoderma*", about a hundred protozoa species and two yeasts are found in cow dung. Seventy bacteria were identified from "Desi" cow dung based on physio-morphological and biochemical characterization, with 32 Gram-negative bacteria (Bhatt and Maheshwari 2019).

6.3.1 *Potential Applications of Cow Dung Microflora*

The cow dung microorganisms have potential applications in agriculture, management of environment and health sector (Fig. 6.1).

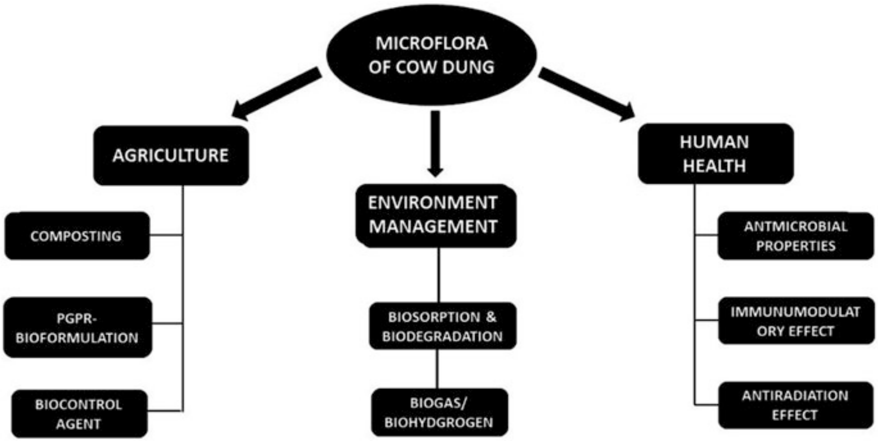

Fig. 6.1 Various applications of the cow dung microflora

6.4 Applications in Agriculture

6.4.1 Composting

Compost is comprised of a number of different elements that are beneficial to soil fertility, pH, microbial diversity, mineral nutrients and temperature sensitivity, among other things.

Composting is a scientific method of making organic fertilizer or manure. Cow dung is gathered in big quantities in suitable containers or large pits seen in Indian village farming fields. This procedure allows the microorganisms to degrade the complex organic matter to simple one or inorganic matter. The collected cow dung is mixed with Panchgavya's formulation and composted for use as an organic fertilizer in agriculture. The compost, which is basically the compost used during seeding the land before planting the crops, is ready to use after 3–5 months (Figs. 6.2 and 6.3).

Fig. 6.2 The cow dung collected in the form of heaps in rural India

Fig. 6.3 Mixing of cow dung with panchgavya for preparing compost for fertilizer

The large population of India has created severe stress on various natural resources. Further, the agricultural dependent countries are highly affected by rapid population growth. Polluted waste is being generated as a result of urbanization and industrialization, which is posing a serious threat to agricultural land. To feed a big population, food production now necessitates the significant use of chemical fertilizers and pesticides, which degrade soil fertility over time while also causing a variety of ailments, including the feared cancer. This chemical fertilizer and pesticide dependency has forced scientists to think about alternative sources of fertilizers.

The microbiota found in cow manure can be utilized instead of chemical fertilizers. They can digest complicated materials into simpler ones and have genetic content that aids in the production of plant growth promoters, amino acids and other nutrients. To increase soil fertility, the entire cow dung is already employed in agricultural land health tonics. It's also studied that microorganisms have a great role in increasing crop yield; their contribution towards soil health is immense.

Microorganisms aerobically degrade diverse organic wastes (substrates) into carbon dioxide and water minerals in composting. Compost (organic manure) can help the soil retain water and increase its nutrition (Vakili et al. 2014). According to several research, adding organic waste to cow dung improves the nutritional content of compost. Microflora's high nutritional content can help minerals be more readily available. Cow dung microorganisms can help boost soil fertility and crop productivity. Cow dung also contains bacteria that aid in the solubilization of Zn, P and K as well as the creation of phytohormones, which are essential for plant growth (Bhatt and Maheshwari 2019). The study by Ofomatah et al. (2021) suggests that the use of rice husk with cow dung forms a residue which is an effective combination for making biofertilizers. Further, the study by Zhang et al. (2020) also suggests that cow manure fertilization increases the diversity of soil bacteria more significantly as compared to chemical fertilizers. It also regulates the structure of the soil bacterial community.

6.4.2 Application in PGPR-Based Formulations

Cow dung includes a wide range of microorganisms that can be combined with urine to create biofertilizers. As a source of beneficial microorganism inoculums, organic farming often uses indigenous formulations based on cow dung fermentation. As previously stated, these preparations contained a wide range of macro- and micronutrients, as well as growth-promoting compounds like IAA and gibberellins, as well as beneficial microbes' acid and alkaline phosphatase activity, which allows insoluble forms of P to be converted to soluble forms in some bacteria found in cow dung (Walpola and Yoon 2012). Many bacteria, fungi and actinomycetes that are P-solubilizing microorganisms can be found in the rhizosphere (Swain et al. 2012; Radha and Rao 2014). Cow dung microorganisms have been found to mobilize insoluble forms of zinc in soil, making them readily available to plants (Bhatt and Maheshwari 2019). Both cow dung and PGPR bacteria stimulate plant growth by managing nutritional and hormonal balance; creating plant growth regulator (PGR) and phytohormones as indole abscisic acid, cytokinins, gibberellins, kinetin and solubilizing nutrients phosphorus and sulphur; and giving infection resistance (Siddiqui and Futai 2009). Ray and Swain (2013) studied the microbiota of cow manure and found that it has an advantage over PGPR.

6.4.3 Application as Biocontrol

The bacteria found in cow dung have antagonistic effects on pathogenic fungi *Fusarium solani*, *Fusarium oxysporum* and *Sclerotina sclerotiorum*, inhibiting conidial germination and mycelia growth, and have antifungal properties similar to penicillin and streptomycin (Basak and Lee 2001; Basak et al. 2002; Girija et al. 2013; Swain et al. 2008; Swain and Ray 2009). The basic mechanisms are the hostile bacteria colonize the surface area quickly and secrete cell wall-disintegrating enzymes (cellulase, chitinase and polygalacturonase) as well as antifungal secondary metabolites (siderophore) to suppress fungal pathogen growth (Swain et al. 2008).

According to Nautiyal et al. (2013) a covering of cow dung on chickpea seeds hindered cell wall-degrading enzymes (hydrolytic enzymes), which operate as a biocontrol for fungal development in *C. arietinum* roots. Patel et al. (2016) found cow dung and urine to be useful in reducing sugarcane red rot caused by *Colletotrichum falcatum*. *B. amyloliquefaciens* and *Streptomyces cochorusii* are strains of *Streptomyces cochorusii* and *B. amyloliquefaciens*, respectively. Yang et al. (2017) reported in cow dung that the *Streptomyces cochorusii* and *Bacillus amyloliquefaciens* have been discovered as potential biocontrol agents for the rice sheath blight pathogen fungus *Rhizoctonia solani*. According to Lu et al. (2014), nematicidal activity against the nematodes *Caenorhabditis elegans* and *Meloidogyne incognita* was discovered in microorganisms from the genera *Proteus*, *Providencia* and *Staphylococcus* isolated from cow dung.

6.4.4 Environmental Health

Cow dung has the potential to become one of the most important auxiliary agents for pollution control, gas reduction and environmental remediation. Cow dung and panchgvya were used in ancient times in Rishis' Hawan and Yajna rites. Hawan and Yajna have long been regarded as effective air purifiers. Mishra et al. (2020) conducted a medico-legal research study which found that Agnihotra yajna, which is performed with refined cow ghee, uses cow dung cakes. Natural items such as grains and ghee are offered to a fire constructed of cow dung cakes in an inverted pyramid-shaped copper kettle during this Yajna. This yajna is said to be a supplementary treatment that clears energy blockages in the meridians and Nadis of the human body, restoring life (Nair 2017).

It can be used to substitute fossil fuels for energy generation in some cases, such as replacing biogas produced from cow dung with LPG for family cooking. On the other hand, it is extremely effective in reducing reliance on biomass burning, particularly in rural and remote places where, on the one hand, LPG supply is difficult and, on the other hand, because to its rising cost, a large number of people rely on it. Cow dung could be a viable remedy for all of these issues, providing an efficient, cost-effective and readily available solution. In addition, we shall go over its application in the context of the environment in further depth below.

6.4.5 Source of Energy

The excessive use of fossil fuels like coal, petroleum fuels, etc. in the industry, energy and transport sectors emits a large number of greenhouse gases that have been identified as a major cause of global warming and climate change. Fossil fuels are also the main source of gases like SO2, NOx, etc. which in the shorter term affect the air quality and hence have a negative impact on human health.

Non-renewable resources are depleting on a daily basis, and various studies have been reported on the benefits of using cow dung in biogas and bio-hydrogen synthesis (El-Mashad and Zhang 2010; Membere et al. 2012). Biogas is produced by anaerobic digestion of organic materials by methanogenic bacteria and contains methane (CH_4) as well as other gases such as CO_2, H_2S, NO and SO. Dung from cows can be used to make a variety of products. Cellulolytic bacteria in cow dung break down the organic elements in manure to a significant level (Gashaw 2016). Hydrolysis, acidogenesis/acetogenesis and methanogenesis are the three phases that lead to biogas production in anaerobic circumstances.

The synthesis of bio-hydrogen using cow dung microorganisms in anaerobic fermentation is well documented. The thermophilic, cellulolytic and hydrogen-producing microbes abound in the rumen microflora (Nissila et al. 2011). Dark fermentation was used to produce bio-hydrogen from a 1:1 mixture of cow dung and food waste, with a yield of 26.9%. Antony et al. (2018) published a report on the subject.

6.4.6 Cow Dung as Building Material and Raw Material for Paper Making

A traditional practice in Indian villages is to apply a mixture of mud and cow dung to the floors and walls of the house. This mixture creates a barrier that protects the house from excessive heat. The fibrous waste from biogas plants can be utilized to make fibreboards, which can then be used to make furniture when mixed with resin.

Cow dung contains a lot of fibre, which can help with paper making and is a process that several Rajasthan regions have adopted.

6.4.7 Biosorption, Bioremediation and Biodegradation

Heavy metals in wastewater and industrial effluents (such as copper, cadmium, lead, nickel, zinc and others) constitute a severe environmental hazard. Cow dung is recognized as a natural and environmentally friendly source of heavy metal ion biosorption (Wang and Chen 2009; Geetha and Fulekar 2013; Gupta et al. 2016). In order to remove Cr VI, dry cow dung powder was used (Barot and Bagla 2012). Rahman et al. (2014) have further reported that cow dung in aqueous solution helps in removal of Arsenic. Arsenic was volatilized from solution and sludge by microorganisms found in cow dung. After 54 h of incubation with Panchagavya, bioleaching of lead to 64% and copper to 49% was observed (Praburaman et al. 2015). In Panchagavya, high-performance chromatography analysis revealed the presence of lactic, malic, acetic, citric and succinic acids, which may be essential components in heavy metal elimination (Rattan et al. 2008). Textile dyes such as methylene blue, blue RGB and eosin YWS have been reported to adsorb in cow dung.

The potential of cow dung microflora to bioremediate hazardous substances such as toluene, benzene, xylene, phenol and other halogenated compounds has been demonstrated (Singh and Fulekar 2009). Degrading enzymes such as cytochrome oxidase and catalase were found in *Pseudomonas putida* isolated from cow dung, which may aid in the effective breakdown of benzene to almost 68% within 12–68 h. *Bacillus* sp. isolated from cow dung was found to be effective at degrading the halogenated chemical (2, 2-dichloropropionic acid) (Smail 2014). *Proteus vulgaris* and *Proteus aeruginosa* detoxify pyrene from cow manure, according to Adebusoye et al. (2015).

6.5 Cow Dung and Its Potential Role in Human Health

6.5.1 Human Health Management

Antiseptic and disease-preventive qualities are found in cow manure (Gupta et al. 2016). Panchgavya's therapeutic properties have been employed to generate a variety of herbal medications (Jarald et al. 2008). Cancer, acquired immunodeficiency syndrome (AIDS) and diabetes are some of the conditions that Panchgavya can help with. Panchagavya's immunomodulatory, immunostimulatory and anti-inflammatory properties have been recorded in Ayurveda (Dhama et al. 2013). Fresh cow dung has been discovered to fight the germs that cause malaria and tuberculosis, as well as having antifungal effects, according to Khan et al. (2015).

6.5.2 Cow Urine as Health Tonic

The Charaka Samhita mentions that cow urine is useful against skin ailments, including leprosy. The cow urine can be used as antibacterial, antifungal, antiviral, anthelmintic, insecticide, antiseptic, wound healing, multidrug-resistant, bio-enhancer, anticancer, antidiabetic, antioxidant and lipid-lowering agent and for its chemotherapeutic potential (Joshi and Adhikari 2019). Sarman Singh (2001) patented the cow urine distillate formulation as a facilitator of availability and activity enhancer for bioactive chemicals such as anti-infective and anticancer medications (in the United States, Patent No. 6410 059/2002).

The biochemical analysis of cow urine identified various important constituents that are necessary to our body physiology, such as sodium; sulphur; nitrogen; minerals; vitamins A, B, C, D and E; manganese; magnesium; silicon; iron; chlorine; enzymes; citric; phosphate; calcium salt; carbolic acid; lactose; hormones; and so on; deficiency of these can be recovered by using cow urine (Kumar 2001; Jain et al. 2010; Mohanty et al. 2014). Cow urine aids in phagocytic activity in the fight against bacterial infections by promoting the production of interleukin 1 and 2 (Chauhan 2004; Singla and Garg 2013), IgA, IgG, IgM and T-lymphocytes (Kumar 2013). Even though 95% of cow urine is water, 2.5% urea and the remaining 2.5% a mixture of minerals, hormones and salts stated above, it is generally regarded as safe (Bhadauria 2002; Mohanty et al. 2014).

6.5.3 Anti-radiation Activity

In India and Russia, cow dung is reported to be used to insulate nuclear power facilities. In India, the Rashtriya Kamdhenu Aayog produced a mobile chip made of cow dung, claiming that it guards against dangerous mobile phone radiation (Barot and Bagla 2012). The anti-radiation action should be studied more so that its applications can be explored further.

6.5.4 Source of Microbial Enzymes

Cow dung's microbial diversity can also produce a variety of industrially important enzymes. Studies showed the potential compounds can be extracted like cellulose, carboxymethyl cellulose and cellulose from cow dung of *Bacillus* sp. Several cow dung microorganisms also produce enzymes such as protease, lipase and esterase lipase (Teo and Teoh 2011). The cow dung can be used as a valuable substrate for enzyme production, such as the production of detergent-stable dehairing protease by *B. subtilis* (Vijayaraghavan et al. 2012).

6.5.5 Source of Cow Dung Paint

Cow dung paint keeps the sun out, preventing evaporation of tens of thousands of gallons of water. The paint stops the water from freezing in the winter by keeping it at a normal temperature. It is also widely available due to its inexpensive cost and long lifespan. The goal of cow dung paint production is to create non-volatile paints that are effective in killing germs and bacteria while also being environmentally friendly (Ghamande et al. 2016).

6.6 Conclusion

Cow dung blooms in a number of ways because it is both helpful and abundant. Cow dung is thought to be used to insulate nuclear power plants in India and Russia. India's Rashtriya Kamdhenu Aayog has developed a cow dung-based mobile chip that shields against harmful mobile phone radiation. Despite its widespread acclaimed use in rituals and applications, cow dung was regarded as a very beneficial substance by ancient civilizations all over the world. A further research and biochemical analysis of cow dung and urine are still required to be considered scientifically proven. Private enterprises selling organic fertilizers, pesticides and Ayurvedic medical formulations are now conducting some research in these fields, but the Government of India needs to step up its efforts to promote dairy and the development of pure Indian cow breeds, as well as products made from cow dung, which has been a neglected, albeit extremely valuable resource.

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Chapter 7
Novel Microorganisms Involved in the Production of Sustainable Biogas Production

Charles Oluwaseun Adetunji, Olugbemi T. Olaniyan, and Ajit Varma

Abstract The higher level of industrialization and recent advances in agricultural activities have led to uncontrollable disposal of wastes. Therefore, there is a need to identify a sustainable biotechnological solution, most especially by exploiting the microorganisms derived from the rumen that could be applied in the management of these agricultural wastes. The presence of rumen microorganisms has been reorganized to perform a functional activity for the effective biodegradation of numerous wastes with biogas representing the final product that is obtained after anaerobic metabolism. The process that happens during the generation of biogas happens through biochemical decomposition of organic matter, generating carbon dioxide and methane. Biogas has numerous advantages such as sustainability, cost-effectiveness, eco-friendliness, and biocompatibility. Therefore, this chapters intends to provide a detailed overview on novel microorganisms involved in the production of sustainable biogas production.

Keywords Microorganisms · Biogas · Anaerobic · Hydrolysis · Acidogenesis · Acetogenesis · Methanogenesis

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_7

7.1 Introduction

Bioenergy production is normally derived from industrial, agricultural, and municipal wastes. Biogas generated is normally composed of 30–45% carbon dioxide (CO_2) and 55–70% methane (CH_4). Biogas is normally stored inside gas holders with grid access (Scherer et al. 2000; Schink 1997; Weiland 2010; Wilkie 2008).

Anaerobic fermentation has been recognized as a natural procedure through which microorganisms portend the capability to transform biodegradable substrate into biogas. This process naturally occurs in river sediments, marshes, and wetlands, as well as in the digestive tract of ruminant animals (Krich et al. 2005; Kushkevych 2016; Barton and Hamilton 2010; Ahring et al. 2001; Ziemiński and Frąc 2012. Santosh et al. 2004; Jackel et al. 2005).

Interestingly, several investigations have been performed on the anaerobic fermentation that could help in adequate recovery of biogas most especially from the agricultural and industrial surpluses (Grothenhuis et al. 1991; Ilyin et al. 2005).

There are several techniques that have been applied in the identification of potential methanogens which include 16S rRNAs (Zeikus 1977). Also, the application of recombinant DNA technology has been identified as a strong methodology that could be applied for the identification of genetic and biochemical regulation of the process involved in methanogens (Zeikus 1977; Amon et al. 2007).

There are numerous factors like accumulation of the toxic metabolic products, temperature, composition of specific groups of microorganisms, and substrate quality that determine the generation of methane. It has been observed that sulfate-reducing bacteria metabolism could lead to the production of hydrogen sulfide as one of the final products (Kushkevych 2013). But it has been observed that hydrogen sulfide is very dangerous, most especially for living microorganism, and portends the capability to prevent enzymes derived from numerous groups of microorganisms (Kushkevych 2013).

Leow et al. (2018) reported that microbes are very useful in waste management and represent a sustainable approach due to their importance in biogas production process. Various microbes such as fungi and bacteria are known to efficiently increase the degradation process and eliminate pathogens. The authors particularly identified cellulolytic microorganisms as a powerful organism that can degrade lignocellulosic components and heavy metals through anaerobic digestion.

This chapter intends to provide detailed information on the usage of microorganisms for effective production of biogas.

7.2 Process Involved in Anaerobic Degradation of Organic Wastes

The process involved in the anaerobic biodegradation of organic wastes entails several microorganisms most especially bacterial species such as methanogenic bacteria which produce CO_2 and CH_4 as their major product of digestion process, hydrolytic, acetogenic, and acid forming (Demirel and Scherer 2008; Nealson 1997).

7.3 Hydrolysis

This stage involves the breakdown of polymerized molecules, which entails numerous insoluble organic compounds such as fats. These compounds are broken down into dimers and soluble monomers, fatty acid monosaccharides, amino acids, proteins, and carbohydrates. This phase is referred to as methanogenesis stage through which these microorganisms apply extracellular enzymes that are derived from hydrolases such as lipases, proteases, and amylases and are generated by suitable strains of hydrolytic bacteria (Conrad 1999; Parawira et al. 2008).

There are some factors that influence the breakdown of organic matter such as diffusion, particle size, pH, production of enzymes, as well as adsorption of enzymes. Biodigestion is normally performed through the action of bacteria including anaerobes of the genera *Enterobacter* and *Streptococcus* (Bryant 1979; Smith 1966).

7.4 Acidogenesis (Acidification Phase)

This stage entails the action of acidifying bacteria, which portends the capacity to convert water-soluble substances. This entails the conversion of hydrolysis products to short-chain organic acids (propionic, pentanoic, formic, butyric, acetic), hydrogen, carbon dioxide, aldehydes, and alcohols (ethanol and methanol). Moreover, it has been observed that the broken down proteins normally lead to the formation of peptides and amino acids that may represent another form of energy for anaerobic microorganisms. Furthermore, it has been reported that the process of acidogenesis may be two-directional as a result of the influence of numerous microorganisms. The major pathways of the breakdown entail H_2, acetates, and CO_2, while the process of acidogenesis product performs an inconsequential function. These new products could be applied as a source of energy and substrate due to the action of these methanogens that portends the capability to utilize these novel product.

Furthermore, it has been observed that buildup of electrons through compounds such as higher volatile fatty acids, lactate, and butyrate propionate could be attributed to the action of bacteria involved in enhancing the level of hydrogen concentration available in the solution. The liberation of hydrogen sulfide and ammonia gas might lead to release of unpleasant smell in this stage of biodigestion (Ntaikou et al. 2010). Typical examples of such microorganisms include *Flavobacterium* sp., *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp., and *Clostridium* sp.

7.5 Acetogenesis

This phase involves the action of acetate bacteria most especially those from the genera *Syntrophobacter* and *Syntrophomonas.* They portend the capability to translate acid phase products into hydrogen and acetates which are utilized by methanogenic bacteria (Schink 1997). It has been documented that *Methanobacterium suboxydans* are responsible for the breakdown of pentanoic acid to propionic acid, while *Methanobacterium propionicum* are involved in the breakdown of propionic acid to acetic acid.

It is highly recommended that a symbiosis action should take place in the presence of autotrophic methane bacteria that possess the capacity to utilize hydrogen together with acetogenic bacteria. This is referred to as *syntrophy* (Schink 1997).

Accordingly, acetates represent a major intermediate product derived from the process of methane digestion. Also, the stage of acetogenesis accounts for about 25% of all acetates and almost 11% of hydrogen that is derived during biodigestion (Schink 1997).

7.6 Methanogenesis

Methane obtained from this process is generated from substrates which are products of some other stages including dimethyl sulfide, acetic acid, methylamine, H_2, methanol, formate, and CO_2 (Verstraete et al. 2002; Griffin et al. 1998; Karakashev et al. 2005).

7.7 Application of Microorganisms for the Production of Biogas

Khalid and Naz (2013) isolated and carried out a characterization on different microbes found in commercial fermenters during biogas production in Gujranwala region. Their study revealed that anaerobic digestion is a sustainable way to manage organic waste, thus generating biogas as a form of electricity. From their analysis, it was demonstrated that non-methanogenic and methanogenic organisms are found in the anaerobic isolates, some of which were reported to be *Methanobacterium formicicum*, *Methanobrevibacter ruminantium*, *Bacteroides fragilis*, *Methanothrix soehngenii*, *Peptostreptococcus*, *Methanothrix soehngenii*, *Methanosarcina frisia*, and *Clostridium difficile*. Also, in the aerobic isolates, some of the microbes found to display significant biogas production are *Micrococcus*, *E coli*, *Bacillus anthracis*, *Burkholderia vietnamiensis*, *Bacillus cereus*, *Bacillus subtilis*, *Corynebacterium amycolatum*, *Salmonella enteric*, *Pseudomonas borbori*, *Enterococcus*, and *Streptococcus bovis.*

Carolina et al. (2019) evaluated different microbial composition as well as the type of microbial inoculum and feedstock type produced during biogas production. The authors identified anaerobic microbial digester from cattle manure, leachate, rumen, and oxidation lagoon water using metagenomics and denaturing gradient gel electrophoresis. The authors showed that different bacteria, archaea, fungi, and eukaryotes were present during biogas production processes and were found to significantly contribute to the high yield of biogas.

Asikong et al. (2016) reported that many microorganisms are involved in the bioconversion of wastes like banana peel, pig dung, and *Telfairia occidentalis* vegetable wastes into biogas. In their study, it was established that the substrates generated from these wastes through anaerobic biodigesters were isolated and screened. The bacteria involved were identified as *Pseudomonas* sp., *Escherichia coli*, *Salmonella* sp., *Bacillus* sp., *Staphylococcus aureus*, *Shigella* sp., *Serratia* sp., *Micrococcus* sp., *Citrobacter* sp., *Klebsiella* sp., *Methanococcoides methylutens*, *Methanothrix soehngenii*, *Methanoculleus bourgense*, and *Proteus vulgaris*, while the fungi screened and isolated were *Penicillium* sp., *Fusarium* sp., and *Mucor* sp. The authors showed that the volume of biogas obtained from this process was very high in yield due to the complementary role of these microbes.

Kovács et al. (2013) reported that biogas production can be improved through bioaugmentation with different consortia of microbes. In their study, it was revealed that *Enterobacter cloacae* and *Caldicellulosiruptor saccharolyticus* culture constitutes the largest number of natural biogas-producing microorganisms.

Theresa et al. (2020) highlighted the significant role of microbes in anaerobic biodigestion of residues through hydrolysis, thus producing value-added products and energy. Also, Fagerström et al. (2018) reported the important role of microorganisms in the sustainable biogas production and anaerobic biodigestion process of low-valued materials into highly valued products and other bio-based resources in circular economy.

Bakar et al. (2018) reported that many agricultural residues like animal excreta and other environmental waste can be converted through microbial biotechnology into value-added products. Thus, in their study, they utilized microbe inoculum to biodegrade hemicellulose rice straw. They therefore suggested that these fungi microbe inoculants displayed a potential role in the industrial bioconversion of many biological wastes into sustainable highly valued products.

Poszytek et al. (2016) revealed that agricultural lignocellulose biomass is digested through anaerobic digestion using a consortium of microbes that can break down plant matter by hydrolysis. Onwuliri et al. (2013) reported that biogas production from cow dung demonstrated the presence of *Bacillus licheniformis*, *Clostridium* sp., and *Escherichia coli*. Gao et al. (2020) reported that yeast can be utilized in the production of biogas and other high value products in a large industrial scale through anaerobic digestion process.

7.8 Conclusion and Recommendation

This chapter has provided a detailed information on the utilization of beneficial microorganisms involved in the production of sustainable biogas. Moreover, detailed information was also provided on the process involved in the anaerobic biodegradation of organic wastes and the involved microorganisms most especially bacterial species such as methanogenic bacteria (hydrolytic, acetogenic, and acid forming). There is a need to create more awareness for most people in developing countries on how they could convert their agricultural waste into the production of biogas. This will go a long way in mitigation of numerous environmental and health hazards derived from uncontrollable disposal of agricultural waste which constitutes a lot of anthropogenic activities and higher level of environmental pollution (Adetunji and Anani 2021a, b; Okeke et al 2021; Adetunji et al 2021a, b, c, d; Adetunji et al. 2020; Olaniyan and Adetunji 2021). Moreover, it will prevent the high amount of money utilized by the government and individuals in the management of agricultural wastes.

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Chapter 8
Bioenergy from Animal Manure

Nadira Naznin Rakhi

Abstract In response to the global increasing demand, livestock and poultry production is on the rise producing a large quantity of manure annually. Therefore, the safe disposal of animal manure is becoming a global concern while utilizing animal manure to produce energy is the best possible solution to both waste disposal and growing energy demand. Manure can be collected from farms of cattle, sheep, horses, and buffaloes along with less commonly llama, alpaca, and camelids as well as poultry farms including chicken, geese, turkey, etc. Different thermal, thermochemical, and biological conversion processes including combustion, pyrolysis, hydrothermal carbonization, gasification, anaerobic digestion, microbial fuel cell, etc. are available to convert manure to energy, e.g., heat or electricity as well as fuels in liquid, solid, or gaseous form. The quality and the quantity of energy or fuel produced depend on the composition, quality, and quantity of manure and the conversion technology used. This waste-to-energy strategy not only is a suitable alternative to depleting fossil fuel but also has an enormous environmental impact. Bioenergy from manure ensures a significant decrease in greenhouse gas emission, reduces air and water pollution, and eliminates the risk of eutrophication and the outbreak of pathogen along with safe disposal of manure. So, considering the growing need of an alternative and renewable energy source, bioenergy production from animal manure should be encouraged more through raising awareness, technological development, and large investments both at national and international level.

Keywords Anaerobic digestion · Microbial fuel cell · Bioenergy · Animal manure · Renewable energy

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_8

8.1 Introduction

Considering the increasing demand for energy in the context of ongoing population growth and advancements in the industry with fossil fuel being the main energy source, per capita energy usage has become a limiting factor for the national development, world economy, and civilization (de Oliveira et al. 2018). Also, this growing demand is urging the replacement of nonrenewable fossil fuel sources (petroleum, natural gas, lignite, coal, etc.) with renewable energy, which is emerging as an inevitable issue in the international policy agenda (Pereira 2014). On the contrary, waste management, especially in agriculture, is gaining global attention in response to the global increase in production and consumption (Qin et al. 2010) which in turn can be a potential source of energy through the conversion of biomass providing a single solution to both current energy demand and waste management crisis. Under this waste management strategy, animal manure is an attractive option, as animal farming will not only contribute to sustainable development through food security, nutrition, and employment, but also animal manure can be utilized for energy generation. Such bioenergy based on national farming practice can specifically be significant for countries dependent on imported fuels and for high altitude arid regions of the world having scarce energy sources (Vankat et al. 2010).

Animal manure derived from livestock and poultry sources are organic matter that can be considered as a potential resource for bioenergy production. The availability and amount of animal manure may vary depending on the type of animal, feeding materials and methods, animal body size and excreta production, breed of the animal, etc. (Abdeshahian et al. 2016). Eventually, manure from pig, poultry, dairy farms, and others can be used in power generation such as electricity or fuel production in the form of solid, gaseous (biogas), or liquid that can be again used as power, heat, or fuel for motive power (Agba et al. 2010). Besides, biogas can be converted into higher value fuels by burning directly or through conversion via different chemical, biochemical, and thermal processes. However, despite such biomass including animal manure being the fourth largest source of renewable energy, traditional energy sources still dominate the global energy sector (Al-Hamamre et al. 2014). Also, energy harvesting from animal manure is mainly limited to burning manure in traditional stoves, especially cattle manure for cooking or heating in different parts of the world (Keles et al. 2017). As a result, this potential energy reservoir supplies only 3% of total energy usage in developed countries, which can be at most 33% in case of developing countries (Al-Hamamre et al. 2014). One possible reason behind such a poor rate might be the rural and labor-intensive nature of the energy conversion process starting from animal manure collection, processing and energy generation due to lack of proper policy, investment, and, mostly, implementation of technologies. So, simple but advanced technologies along with traditional practices such as combustion, gasification, pyrolysis, anaerobic digestion, microbial fuel cells, etc. can be employed for energy production from animal manure in terms of higher efficiency and growing energy demand.

Apart from offering unlimited availability and inexpensive resource, the use of animal manure for energy has an enormous impact on the environment decreasing both air and soil pollution linked to waste disposal (Monteiro et al. 2011). According to a report in 2002, United States alone generates approximately 230 million tons of animal waste; the quantity should have increased manyfold in the present time due to the recent growth of livestock and poultry industries (Sheffield 2002b). So, unsafe and improper disposal of such a huge amount of animal waste produced at a global scale may cause major environmental pollution including odors, dust, surface and groundwater contamination, ammonia emission, etc. Moreover, the unplanned use of manure as fertilizer to avoid such environmental problems may also cause damage to water bodies by the eutrophication phenomenon. Besides, conversion of manure into bioenergy may also help stop global warming, as the conversion of animal manure and slurries in biogas reduces greenhouse gas emission, particularly ammonia and methane (Nielsen et al. 2002).

So, considering the progressive depletion of conventional fossil fuel followed by concomitant international conflict regarding energy security and supply, animal manure can be a sustainable source of energy. Also, along with biogas, the popular bioenergy form with higher heating value than natural gas (Serio et al. 2002), other forms of energy with high energy potential can be produced and supplied at a larger scale with the advent of current technologies.

8.2 Types of Animal Manure, Its Composition, and Availability

In recent times, livestock and chicken farming have bloomed because of the growing demands of dairy products, meats, eggs, leather products, and other by-products. But the manure produced does not always represent the manure collected or available for manure utilizing facilities, especially in case of animals not raring in total confinement. So, the term “collectable animal manure ratio” has been introduced to express the available manure for utilization. The commercial animal production system usually keeps the animal in a confined environment, from which all the manure produced can be collected. But such practice is highly absent in small farms or homesteads. So, animals spend part of their lives outside. As a result, it’s impossible or difficult to collect the manure deposited out in the open fields, e.g., pasture areas. So, it makes the prediction of global manure production and availability for energy conversion more difficult, although different data of animal farming practice and manure production at the national level are available. For example, according to a report in 2009, Spain holding the third position among European Union countries in the livestock sector had approximately 6.4 million bovines (7% of the total production of EU), 22.7 million sheep (19%), 2.9 million goats (22%), 24.8 million pigs (15%), 158.2 million poultry (15%), 2.4 million rabbits, and 292,000 horses (Gómez et al. 2010). On the other hand, the manure production rate of an animal can be predicted depending on the type of animal, age of the animal, feed content and its digestibility, etc. On an average, the fresh weight of manure produced by an animal is almost equal to the weight of feed consumed, and its dry matter represents approximately 20% of the feed consumed (Henuk and Dingle 2003). In 2006, Kershaw County in South Carolina, USA, alone produced over 80,000 tons of manure collected primarily from chicken farms followed by turkey farms (Flora and Riahi-Nezhad 2006). So, such observations can be used to roughly estimate the available manure at the global or the national level.

The common animals, usually domesticated animals that can be counted for manure-based bioenergy production, include cattle, goats, poultry (hen and turkeys), swine, etc. along with the less common ones such as horses, llamas, alpaca, camelids, etc. For the ease of manure production estimation, the common animal sources can be divided into two groups as livestock (different ruminant, e.g., cattle, sheep, goats, etc., and non-ruminant animals, e.g., pigs, etc.) and poultry (hens, turkeys) group. The manure production of these two groups focuses only on the most common animals for manure production and utilization as biofuels.

8.2.1 Livestock Manure Production

This category includes different large ruminant farm animals such as buffaloes, cattle, etc. and small animals such as sheep, goats, etc. The excreta production depends on the animal type, age, and body weight (Afazeli et al. 2014; Avcioğlu and Türker 2012). On average, the daily manure production can be estimated as 10–20 kg for cattle and 2 kg for sheep and goat which is about 5–6% and 4–5% of their body weight, respectively (Avcioğlu and Türker 2012). Other studies calculated manure production based on the 9% of the body weight of the large ruminant and 4% of the body weight of small ruminants (Afazeli et al. 2014). So, if the average body weight of the large and small ruminant animals is 250 kg and 40 kg, respectively, on average, their daily manure production can be estimated as 22.5 kg and 1.6 kg, respectively (Afazeli et al. 2014). However, along with manure, blood and rumen content of slaughtered animals are also the potential animal waste for energy production, especially biogas in the anaerobic digester; blood accounts for 8.4% and 3% of the body weight of large and small ruminant animals, respectively; and rumen content accounts for 12% and 25% of the body weight of large and small ruminant animals, respectively (Afazeli et al. 2014). However, this estimation may vary depending on age and the types of animals, even between dairy and beef cattle and so on. Also, the manure quantity may vary depending on the concept of collectable manure. Table 8.1 represents daily manure production and collectable manure ratio of different animals (Ekinci et al. 2010).

Table 8.1 Estimated animal manure production rate and collectable manure ratio

Type of animal	Manure production per animal (kg/day)	Dry matter content (%)	Volatile matter content (%)	Collectable manure ratio
Dairy Cattle	43	13.95	11.63	0.5
Beef Cattle	29	14.66	12.41	0.5
Sheep	2.4	27.5	23	0.13
Goats	2.05	31.71	23.17	0.13
Horses	20.40	29.41	19.61	0.29
Broiler chickens	0.19	25.88	20	0.19
Poultry chickens	0.13	25	18.75	0.13
Turkeys	0.38	25.33	19.36	0.26
Ducks and geese	0.33	28.18	17.27	0.22

Table 8.2 Estimated manure production by broilers, replacement pullet and layers; fresh manure are based upon feed consumption according to a report in 2002 (Bell et al. 2002)

Birds (n = 1000)	Fresh manure—average tons/day	Fresh manure—average tons/year	Dried—average tons/year
Broilers			
To 42 days	0.87	237.2	79.1
To 49 days	1.01	287	95.6
To 56 days	1.14	332.8	110.9
Replacement pullets (to 20 weeks)			
White egg type	0.54	179.4	59.8
Brown egg type	0.61	200.6	66.9
Layers			
White egg type	1.13	410.6	136.7
Brown egg type	1.28	465.4	155.1

8.2.2 *Poultry Manure Production*

The poultry category includes different types of Chickens, Ducks, Geese (Layer or broiler), etc. On average, such animals produce manure of approximately 3–4% of their body weight, e.g. for chicken, 0.08–0.1 kg/day (Table 8.1) (Avcioğlu and Türker 2012; Kaygusuz 2002). However, the quantity may vary depending on their age, body weight, feed consumption etc. (Table 8.2).

However, manure production by hen can be highly influenced by feed consumption and can be 35%–145% of their feed consumption (Bell et al. 2002). The fresh weight of daily manure produced by hen may be equal to the amount of feed consumed. For instance, daily manure of white leghorns is approximately 140–195 g, when it was collected in oil pans to prevent evaporation of water, which was about 1.45 times the amount of feed they consumed. On the other hand, the daily manure of the average hen would be 122 g accounting for almost equal to their feed consumption; but it represented only 35% of the feed consumed when manure was not collected in oil pans and dried in high-rise layer houses not being collected immediately (Henuk and Dingle 2003).

However, the annual growth rate for the production of beef and dairy cattle is estimated to be 0.32% and 0.33%, respectively, while pork and poultry are assumed to have a rate of 0.47% and 1.24%, respectively (Meyer et al. 2018). So, the current number of slaughtered animals registered by Eurostat can be used to assume the animal manure potential in the year 2030 or later at least in the EU countries.

8.2.3 Compositional Diversity of Animal Manure

The mineral composition or the nutrient content of animal manure may vary with the type of animal depending on feed content, digestibility of the ration, age of the animal, and other factors (Shen et al. 2015), although similarities exist between dairy and beef manure, or between layer and broiler manure among chicken with pig manure showing better homogeneity than other types of manure (Shen et al. 2015). However, such variation in composition needs to be taken into account in the design and operation of energy conversion processes considering their differential effects on the process and its equipment. For example, the level of S in animal manure should be considered in the design of the gasification plant, as S in manure converts to H_2S which is highly corrosive capable of causing serious damage to syngas coolers and eventually to the heat exchange system (Virmond et al. 2012). On the other hand, the high concentration of methane gas in cattle manure makes it a suitable fuel for heat generation and even spark-ignition engines (EMBRAPA 2003). So, the composition analysis of the manure should be the first and significant step of manure handling and utilization facilities.

The mineral composition of animal manure shows extremely large variations, primarily due to variations in feed additives used in animal diets. Focusing on the eight common mineral elements (K, Ca, Na, Mg, P, Fe, Cu, and Zn), differences in concentration of those elements were reported as below in major types of animal manure (Shen et al. 2015):

1. Pig manure: P > Ca > K > Mg > Fe > Na > Zn > Cu
2. Dairy and beef manure: Ca > K > Mg > P > Fe > Na > Zn > Cu
3. Layer and broiler manure: Ca > K > P > Mg > Na > Fe > Zn > Cu

According to different studies, pig manure contains a significantly higher concentration of P, Mg, Zn, and Cu, among which, P, Cu, and Zn content show the highest variability, while excessive Cu and Zn in pig manure usually arise due to the overuse of feed additive (Li et al. 2007). On the other hand, despite the striking similarity in the concentration order of different elements between dairy manure and beef manure, a distinct difference in the level of Fe may be noticed. The soil incorporated in the dairy manure results in such a high level of Fe compared to beef manure (Shen et al. 2015). The K, Ca, and Mg contents show the highest variability in layer manure with the highest level of Ca content, almost twofold higher than any other animal manure (Shen et al. 2015). Such a level of Ca is also associated with their feed content of the animal rich in flour. However, alkali metals such as Na, K, etc. and S were found rich in layer manure, broiler manure, and pig manure, which indicates the risk of corrosion during combustion due to sulfation reaction (Obernberger et al. 2006). Besides, a high alkali metal is considered a challenge in producing fuel from animal manure, because the higher level of alkali metal in animal manure leads to lower melting temperature hence poorer fuel performance in the thermal conversion unit (Virmond et al. 2012). To express the level of alkali metal in manure, alkali index (AI) is used, which means the quantity of alkali oxide in the fuel per unit of fuel energy (kg alkali/GJ) and considered as the threshold indication for slagging and fouling (Jenkins et al. 1998; Akinrinola et al. 2014). AI can be expressed as below:

$$\mathrm{AI} = \mathrm{kg}\ (\mathrm{K_2O} + \mathrm{Na_2O})/\mathrm{HHV}$$

Here, HHV is the higher heating value of animal manure per kg and calculated as $\mathrm{HHV} = \mathrm{GCV} - 0.094283 \times \mathrm{S}$, while GCV (MJ/kg) indicates the gross caloric value of animal manure per kg and S (%) represents the total sulfur content of animal manure per kg. The AI value exceeding 0.34 would be considered risky, and high AI value of manure indicates severe fouling and ash deposit problems (Thanapal et al. 2012). Dairy and beef manure has lower AI index values than other types of manure. However, the ash content remaining after burning or combustion and decomposition is another significant parameter to be considered for utilizing the leftover elements in the manure after the energy conversion process.

More importantly, H/C and O/C ratios of manure are key parameters to evaluate the suitability of manure in energy production and reactivity (Tao et al. 2012; Chiang et al. 2012; Fernandez-Lopez et al. 2015). That is because the chemical bond energy between carbon–oxygen and carbon–hydrogen is lower than the bond energy of carbon–carbon. So, the increase in the level of H and O will contribute to a decreasing heating value. Besides, the H/C and O/C ratios indicate the reactive characteristics of animal manure. So, high H/C and O/C ratios of manure (e.g., broiler manure) mean high volatility of manure during thermal conversion processes including gasification and pyrolysis. However, the H/C and O/C ratios of animal manure are higher compared to coals and biochar, and among different types of manure, O/C ratio of pig manure and H/C ratio of beef manure have lower values than other types of animal manure with higher HHV values, making them suitable candidates for thermal conversion processes (Shen et al. 2015). On the other hand, C/N ratio of animal manure is significantly lower than plant biomass, which affects the energy conversion performance of anaerobic digestion (Almomani et al. 2017). The C/N ratio needs to be maintained at an optimum level for biogas production through anaerobic digestion. Because a very high C/N ratio results in rapid consumption of nitrogen by the bacteria in the anaerobic digester leaving the carbon not capable of reacting, a very low level of C/N ratio causes the rapid conversion of nitrogen to ammonia causing the pH of the substrate biomass to be increased to a level toxic to the bacteria. Ultimately, methane or biogas production decreases in either way (Appels et al. 2008; Lay et al. 1997).

In general, the ash content and the mineral contents of cattle manure differ significantly from poultry manure, and cattle manure has lower ash and mineral element content than other animal manure. So, cattle manure is more suitable for the combustion and gasification process than other animal manure. But complete compositional analyses including both proximate and ultimate analysis of animal manure along with the thorough evaluation of the animal manure availability and quantity need to be completed before establishing bioenergy production facilities from animal manure.

8.3 Energy Conversion Processes

Different thermal, thermochemical, and microbial processes make it able to extract the energy stored in animal manure. The conversion processes include direct combustion or combustion with other wastes or fossil fuel, dry and wet gasification, pyrolysis, anaerobic digestion, etc.

Direct combustion of solid manure may be used to produce heat or electricity, and the efficiency of electricity production may be enhanced by co-firing with other waste or fossil fuels such as coal. The most commonly used energy conversion process is anaerobic digestion to produce biogas, primarily composed of methane, which has a significant impact in reducing odor and greenhouse gas emission apart from supplying energy. More importantly, biogas can again be utilized for secondary power generation in the forms of electricity or heat and can be used as a boiler or furnace fuel or to run refrigeration units. Besides, use of modern techniques and technologies such as sterling engines, microturbines for the biogas (about 30%), and fuel cells (40% or more) increases the typical efficiency of energy generation (Sheffield 2002a). However, liquid fuels can also be produced from biogas or directly from manure.

The energy conversion processes are described briefly below.

8.3.1 Combustion

Combustion of animal manure is a thermal and one of the most primitive methods for energy extraction, in which manures are directly burned to generate heat or process for small-scale operations. For large-scale operations, electricity can be produced through combustion using a boiler to burn fuel and a steam turbine to convert the motive force into electricity. Also, this is especially economical while operated at a large scale, specifically, if the manure production facility is not far away (Flora and Riahi-Nezhad 2006). However, most fresh manures usually contain too high moisture to be burnt. So, before combustion, manures are dried first. Poultry manure is compatible with combustion having a heating value between 6 and 8 MJ/kg. A combustion plant may yield electricity with about 28% efficiency, although lower than that of comparable coal-based plants due to the sticky nature of the ash and corrosiveness of the flue gas which causes the steam temperature in the boiler limited. That means that the poultry manure combustion will be higher per kWh of electricity produced. Combustion in a furnace is commonly applied; but grate furnace incinerator, a fluidized bed incinerator, or a rotary kiln can also be used (Van Caneghem et al. 2012). However, the most important implication of such manure-based combustion unit is rather safe disposal of the manure, as animal manure contains different pathogens threatening the emission of these pathogens to air and water upon land spreading (O'Connor et al. 2005). But the high turbulence of a fluidized bed combustor will cause efficient heat transfer and uniform mixing destructing the pathogens in manure (Van Caneghem et al. 2012). Moreover, land application has not only the risk of pathogen emission but also has the risk of eutrophication in the water bodies through emission of nitrate (NO_3), ammonium (NH_4), organic N species (e.g., urea or uric acid), and organic C. The environmental impact caused by these emissions is mainly eutrophication as discussed later. So, undoubtedly, the combustion of manure has a low environmental impact apart from energy supply.

8.3.2 Pyrolysis

Pyrolysis, a thermochemical energy conversion process, is a potential alternative to incineration or combustion, especially for chicken manure due to its low ash and moisture content along with wide availability (Serio et al. 2002). Two-stage reactor facilitating primary and secondary pyrolysis may produce a medium Btu fuel gas from animal manure, although energy conversion through pyrolysis initially focused mainly on plant biomass instead of animal manure (Elliott et al. 1991; Rowell 1984; Klass 1998; Serio et al. 2002). However, staged pyrolysis to produce medium Btu fuel gases (350–550 Btu/ft^3) from animal manure offers several advantages over conventional anaerobic digestion (Serio et al. 2002). It has higher process throughput, produces higher btu fuels, produces much less NOx from manure nitrogen, and needs much less water than the anaerobic digestion (Fig. 8.1). Also, this can be operated with poultry litter which is not a suitable candidate for anaerobic digester and does not convert as much of the manure nitrogen to NOx.

To produce pyrochar from animal manure, manure is dried at 105 °C and milled to pass through a screen of 2 mm particle size and rehydrated the manure to produce the ultimate 6 mm pellets (moisture contents of 20−30%). Those pellets can be pyrolyzed in the following four steps (Ro et al. 2018):

1. Holding for 60 min and purging using industrial-grade N_2 at 200 °C for equilibration
2. Ramoing up to the purolytic temperature while dropping N_2 flow within 60 min

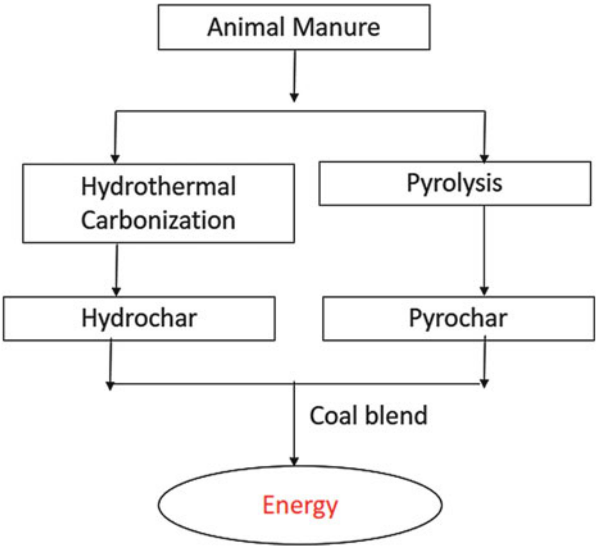

Fig. 8.1 Hydrochar and pyrochar for energy generation

3. Again holding for 120 min for equilibration at the desired temperature
4. Cooling to below 50 °C followed by purging before withdrawing the pelletized biochar samples

Pyrolysis and hydrothermal carbonization, another modified version utilizing pyrolysis, can convert animal manure into pyrochar and hydrochar, respectively, which can be further converted into energy.

8.3.3 Hydrothermal Carbonization

Among the emerging technologies for higher energy values and ash contents, hydrothermal carbonization (HTC) is an attractive option to produce energy in the form of char from animal manure, which can be utilized to produce heat and power through combustion. The carbon-rich char produced after HTC is referred to as hydrochar, which can be produced from wet feedstock requiring less energy than conventional dry pyrolysis (Libra et al. 2011). One important advantage over conventional pyrolysis or combustion is that HTC does not require prior drying and is a low-energy waste treatment technology, especially suited to wet manure (Berge et al. 2013). The HTC reactions are operated relatively at occur low temperatures (180–250 °C) in a closed system in the presence of water or steam under autogenic pressures. The hydrochar produced is rich in carbon and ash content with high energy potential, making it a competitive carbon forms as fuel (Li et al. 2015; Li et al. 2007) (Fig. 8.2).

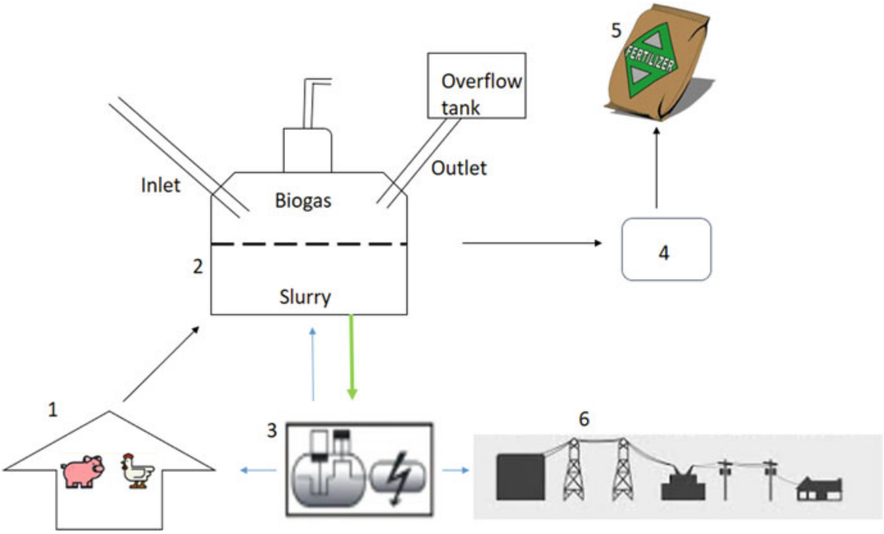

Fig. 8.2 Biogas plant using animal manure. (1) Livestock and poultry farms, (2) anaerobic digester, (3) power and heat cogeneration, (4) post-treatment tank, (5) collection of leftover from the digester as fertilizer, (6) energy distribution grid

Hydrochars produced from swine solids or poultry litter can be produced using two feedstock solids contents (20% and 50% total solid) with two HTC temperatures (200 and 250 °C) and two reaction time periods (4 and 20 h) in case of swine solid hydrochars and 250 °C for 20 h of reaction time in case of poultry litter hydrochars (Ro et al. 2018). Both the hydrochars contain high ash contents than fossil coal samples having a high energy content higher than the respective feedstocks. Both peak and burnout temperatures of hydrochars are lower than fossil coal samples implying that hydrochars are easier to ignite than coal and converted completely at shorter residence time and lower temperature, respectively (Ro et al. 2018).

8.3.4 *Gasification*

Gasification is another thermochemical conversion process to produce bioenergy. The first step of this process is pyrolysis at temperatures under 1100 °F to produce char or fixed carbon from animal manure and to vaporize the volatile compounds. So, in the next step, those char and volatile products will be combusted with O_2 to produce heat for gasification along with producing CO and CO_2. The third and final step is the gasification step, in which the char reacts with CO_2 and steam to produce CO and H_2 (syngas). The latter products (CO and H_2) are the desired outcome of gasification, which will be fired directly in a gas turbine for power generation or chemical synthesis. Gasification utilizing poultry litter at a small scale has been successful (Reardon et al. 2002), and even a large-scale production plant based on poultry litter worthy of $20 million has been being operated in Carneville, USA, for electricity (Mukhtar et al. 2002).

8.3.5 Anaerobic Digestion

Biochemical degradation of organic matter including animal manure, primarily by microbial action to produce methane, known as biogas is a promising alternative energy source considering its energy values, environmental impact, and sustainability. Moreover, biogas can be burnt as fuel or can be converted to fuels with higher energy values and electricity (Fig. 8.3). Biogas is a flammable gas with no color or odor and typically consists of methane, carbon dioxide, and hydrogen along with trace amounts of other particulates and contaminants (Lyytimäki 2018). Although the ratio of major gaseous components of biogas varies depending on the raw material and method of production, methane and carbon dioxide, the two major

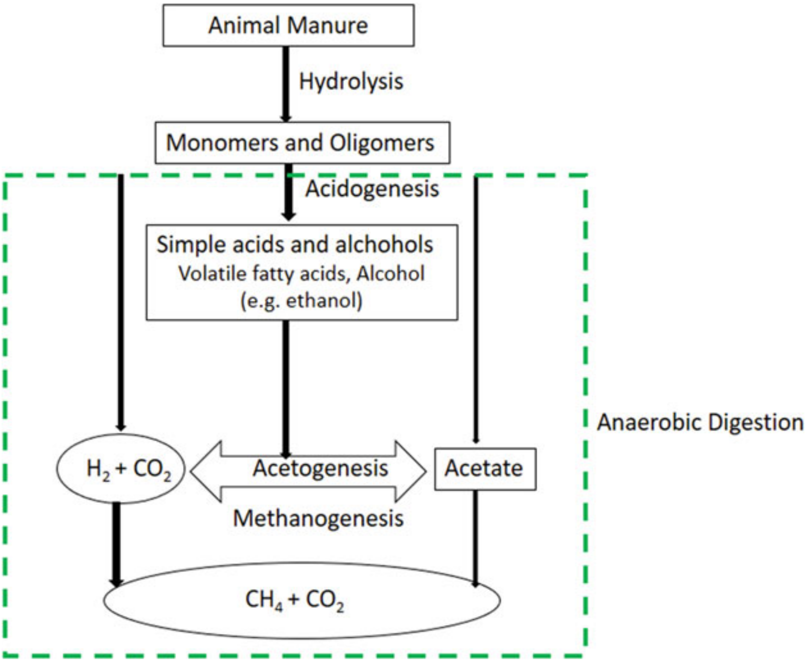

Fig. 8.3 Three stages of anaerobic digestion of animal manure for biogas production. The first stage is hydrolysis which may or may not be necessary depending on the starting materials and need not be done in anaerobic condition. Next two stages are acetogenesis and methanogensis operated in anaerobic digester, and the final product is biogas, primarily composed of CO_2 and CH_4

Table 8.3 Typical composition of biogas (Lyytimäki 2018; Holm-Nielsen et al. 2009)

Components	Amount (%)
CH_4	50–70
CO_2	25–50
N_2	0–10
H_2	0–0.1
H_2S	0–0.3
O_2	0–0.2
Calorific value	21–24 MJ/m^3

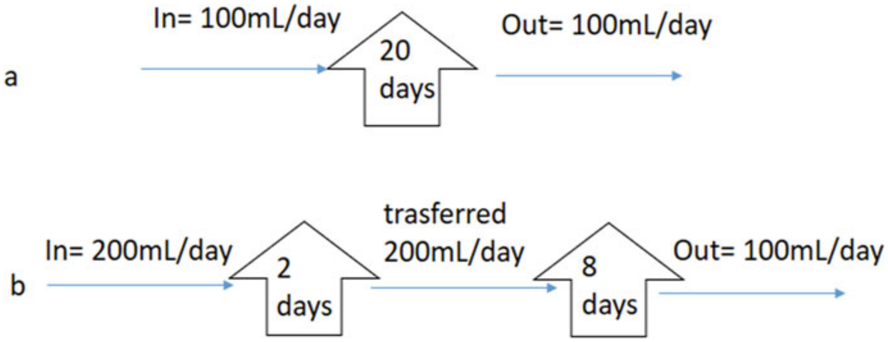

Fig. 8.4 Experimental setup of single-stage (**a**) vs two-stage (**b**) anaerobic digestion according to Demirer and Chen (2005). Retention and volume differ in these two experimental setups. While the tank is of 2000 mL volume in case of (**a**) with 20 days of retention time, first tank in (**b**) has 400 mL volume with 2 days of retention time, and the second tank has 1600 mL volume with 8 days of retention time

components, remain in the range of 40–70% and 15–60%, respectively (Rodriguez et al. 2017). Typical composition of biogas is presented in Table 8.3.

Biogas production is possible from nearly all types of biological feedstocks, among which animal manure and slurries from cattle, pigs, poultry, and even fish, fur, etc. represent the largest resource (Holm-Nielsen et al. 2009). The widely known method of biogas production is anaerobic digestion, which is operated into three stages as described by Themelis and Ulloa (2007) (Fig. 8.4).

Hydrolysis is the first step in biogas production to convert complex organic solids and polymeric macromolecules to simple soluble molecules that microorganisms will be able to utilize and metabolize. Hydrolysis is not part of the anaerobic process; rather microorganisms work on hydrolyzed animal manure to produce methane in the later reactions in the anaerobic digester. Although hydrolysis is not mandatory for all types of feedstocks, it is significant for certain wastes including animal manure (Li et al. 2015).

In the second stage, those soluble molecules are converted to simple organic acids, carbon dioxide, and hydrogen. Organic acids such as acetic acid, propionic acid, butyric acid, and ethanol are produced in this step. Methane (CH_4) is produced at the last stage during methanogenesis. Microorganisms that carry out this methanogenic conversion are called methanogens and are in the domain of Archaea. These methanogens are of two types: hydrogenotrophic and acetoclastic methanogens. Hydrogenotrophs works on H_2 and CO_2 to produce methane, while acetoclastic methanogens convert acetate into methane.

$$\text{Hydrogenotrophic methanogens}: CO_2 + 4H_2 = CH_4 + 2H_2O$$

$$\text{Acetoclastic methanogens}: CH_3COO^- + H_2O = CH_4 + HCO_3{}^-$$

Although the second step can be operated at a wide range of temperature and pH, methanogens are sensitive to fluctuations of environmental parameters such as temperature, pH, retention time, etc.

Anaerobic digesters are large fermenters with mechanical mixing, heating, and gas collection and can be different types based on their mode of operation, such as:

- Batch
- Semi-continuous
- Continuous

The reactor type for biogas production should be chosen depending on the waste characteristics, particularly the particle solid contents or total solids. Continuous flow stirred tank reactors are suitable for high total solid feedstocks and slurry waste, while soluble organic wastes should be treated in high-rate biofilm systems such as anaerobic filters, fluidized bed reactors, and upflow anaerobic sludge blanket reactors (Angelidaki and Ellegaard 2002). Also, depending on the raw material used for anaerobic digestion, the digester size may vary (Karellas et al. 2010). For example, for animal manure such as cow manure, or the smallest size plants, horizontal digesters of 50–150 m^3 volume will be suitable. Also, such design is well equipped to support the co-digestion of plant biomass and animal manure due to good mixing. Apart from such small vessels, other larger volume digesters, such as upright standard agricultural digester (500–1500 m^3) and upright large digester (1000–5000 m^3), are available for anaerobic digestion.

The efficiency of digesters depends on factors associated both with manure composition and operation conditions such as quality of manure, the temperature of the digester, retention time in the digester, etc. Even the duration between manure collection and anaerobic digestion affects the biogas production efficiency. A study in 2006 reported such phenomenon of decreased biogas production while the manure was deposited in a pit for 4–5 months before transferring to the anaerobic digester (Jenner 2006). Temperature is another important parameter for the performance of the anaerobic digester affecting the rate of digestion and CH_4 production (Ward et al. 2008). On average, microorganisms can be classified as psychrophile preferring the temperature lower than 20 °C, mesophile preferring temperature within 20 °C–45 °C, and thermophiles with temperature preference in the range of 45°–60 °C. Anaerobic digester is mostly operated at a temperature of mesophiles or thermophiles, although there is no standard temperature zone for anaerobic digestion due to conflicting results with both the temperature zones (Monteiro et al. 2011). However, mesophilic organisms showed high tolerability to toxicants and temperature fluctuation compared to other classes of organisms (Nguyen et al. 2007). So, the digester operated at mesophilic temperature is more stable and thus popular.

Biogas potential is expressed as % of total solids and calculated using the average yields from the anaerobic digestion process of volatile solids. So, the potential differs based on the composition of the manure or, more specifically, the animal species from which manure was collected (Tricase and Lombardi 2009). Manure collected from ruminant animals, especially cattle manure, is very much compatible with the fermentation process, as it already contains methanogenic bacteria. But the biogas produced from cattle manure is lower than those of chicken and pigs, because cattle absorbs comparatively higher percentage of nutrients from fodder and also, the leftover lignin complexes from high-fiber fodder resist the reaction of anaerobic fermentation (Monteiro et al. 2011). If the biogas yield coefficient, which is specific for a specific animal, can be determined as done by the probiogas project, the biogas production potential of an animal of species i and type j can be calculated using the formula below (Ramos-Suárez et al. 2019):

$$B_{\mathrm{h}} = \sum_{h} A \times Q \times \mathrm{TS} \times \mathrm{VS} \times Y$$

Here, B_{h} means the daily biogas volume produced in the livestock farm h (m^3/day), A means the number of animals of the species i and type j in the livestock farm h, Q means the daily manure produced from that type and species of animal, TS is the total solids in manure Q, VS is the volatile solid content in manure Q (%/100), and Y is the biogas yield for manure Q ($\mathrm{L}_{\mathrm{biogas}}\ \mathrm{kgVS}^{-1}$).

8.3.5.1 Measures to Improve the Efficiency of Anaerobic Digestion

(a) Substrate Modification

Co-digestion of Feedstocks

Simultaneous digestion of a mixture of animal manure and suitable organic waste may increase the bioconversion rate and methane yield (Alvarez and Lidén 2009). Such a mixture of substrates facilitates an improved balance of nutrients and rheological qualities of the substrates, decreased effect of toxicants on the digestion or fermentation, etc. (Mata-Alvarez et al. 2000). Besides, considering the economic and the environmental point of view, co-digestion of feedstocks offers significant advantages over conventional methods (Hjorth et al. 2009).

However, co-digestion of animal manure and water hyacinth, a widely available plant with high reproductive capability, can be an attractive solution for many countries not only as a potential renewable energy source but also to convert notorious invasive plant biomass into energy. The highest biogas production, which was higher than biogas produced from pig manure alone, was noticed when 15% of the feedstock was water hyacinth (Lu et al. 2010). Because the C:N ratio of such mixture is optimum for accelerating the growth of fermentation bacteria. Thus, it leads to higher biogas production (Singhal and Rai 2003). Also, water hyacinth is rich in different metals such as Fe^{3+}, Zn^{2+}, Co^{2+}, and Cu^{2+} facilitating the fermentation for higher production of biogas (Patel et al. 1993). But with the higher concentration of hyacinth, more than 15% of the feedstock increases lignin content to an extent that decreases biogas production (Lu et al. 2010).

(b) Process Modification

Ozonation Step

An additional chemical oxidation step such as ozonation in case of two-stage anaerobic digestion increases the efficiency. Only a low dose of ozonation, e.g., 1 mgL^{-1}, increases efficiency of anaerobic digestion and methane recovery (Almomani et al. 2017). Also, higher efficiency and shorter digestion period achieved through this process indicate a significant reduction in the required digester volume, making the operation more economical.

Dry vs Wet Anaerobic Digestion

Dry anaerobic digestion is referred to the system using raw materials $>15\%$ total solids, while wet anaerobic digestion uses waste $<10\%$ total solids. Dry anaerobic digestion offers several advantages over conventional wet anaerobic digestion. For example, dry anaerobic digestion requires reduced size of the fermenter compared to wet anaerobic digesters. Also, the dry technique yields less wastewater compared to wet digestion. In a study, pig manure especially performed well in dry anaerobic digestion compared to poultry or cattle manure, as poultry/cattle manure showed high volatile fatty acid accumulation and pH drop during the operation (Ahn et al. 2010). But changing the design of the digester may improve the situation. However, dry operation is especially recommended while producing biofertilizer from animal manure (Ahn et al. 2010).

Two-Phase Digestion

In a conventional anaerobic digester, both the acidogenesis and the methanogenesis steps take place in the same vessel, while the acidogenic and the methanogenic microorganisms differ in terms of their nutritional and pH preference along with their physiology, growth, nutrient uptake, and other qualities. So, the conditions favorable for acidogenic bacteria, e.g., low pH, may inhibit the growth of methanogens. As a result, in the conventional one-phase anaerobic digestion, pH and organic loading rate need to be adjusted according to the slow growth rate of methanogens, which ultimately slow down the growth of fast-growing methanogens and thus the process as a whole (Massey and Pohland 1978). So, separating these two phases of anaerobic digestion offers additional advantages over the conventional process (Fig. 8.4). Firstly, it facilitates the optimum growth of each bacterial group in different phases, in which complex organic materials are degraded by acidogenic bacteria in the first phase and those degraded simpler organic materials are converted + to desired methane gas in the second phase by the action of methanogens. Secondly, it protects methanogens from pH shock, while low pH, high organic load favors the growth of acidogens but prevents the growth of methanogens in the first step. Also, this modification makes the process smaller and cost efficient (Pohland and Ghosh 1971; Solera et al. 2002; Fox and Pohland 1994). Even in case of dairy manure, two-stage digester produces at least 50% more biogas than one-stage anaerobic digester (Demirer and Chen 2005).

However, biogas production technology should be in focus considering its multipurpose uses, as it can directly be used as energy or can be utilized for secondary energy generation. The lowest value chain utilization of biogas is for the production of heat and/or steam. Even it can be used as an industrial energy source for heat, steam cooling, and/or electricity or for producing chemicals and/or protein. Biogas plants based solely on animal manure produced 12.4% of the total electricity coming from biogas in 2016 (Daniel-Gromke et al. 2018). However, biogas can also be upgraded for use as vehicle fuel or as fuel in fuel cells (Kristensson 2007). Upgrading and injecting biogas in the national gas grids make energy supply easier and economical from the production site to the populated city zones.

8.3.6 Microbial Fuel Cell for Electricity

Animal manure can directly be employed for electricity generation, which is getting attention in recent times. A study demonstrated electricity generation directly from animal manure using a mixture of animal manure and sea sand at a 1:1 w/w ratio in plastic cups containing a cathode and an anode connected (El-Nahhal et al. 2020). Such 50 cups were serially connected forming an environmental battery, and this setup produced electricity with high voltage after 96 h of installation. This environmental battery showed a life of 40–60 days of sustainable electricity generation. Also, they reported the electricity generation potential of animal manure was higher than activated sludge with the following order of potential horse manure > chicken manure > cow manure > sludge, although the maximum electricity output was reported using activated sludge mixed with cattle manure slurry followed by cattle manure slurry alone. However, such setups of electricity generation depend on the action of microorganisms, which is the basis of microbial fuel cells (MFC) for electricity generation.

MFC is a promising technology for sustainable, clean energy generation using microorganisms to convert the chemical energy of organic compounds into electricity. A typical MFC has anode and cathode chambers, usually separated by a cationic membrane. Microbes are in the anode chamber, where they metabolize the organic material acting as the electron donor. Microbial activity produces electrons and protons in this chamber, from which electrons are transferred to the anode surface and finally, electrons move from anode to cathode through the electrical circuit. Meanwhile, protons move through electrolytes followed by the cationic membrane. So, the cathode consumes electrons and protons by reducing soluble electron acceptor, e.g., oxygen (Rabaey et al. 2004). A load is placed between the two electrode chambers to harvest the electrical power (Allen and Bennetto 1993). However, instead of using metal cathodes, the use of biocathode utilizing microbes as catalysts for assisting electron transfer has been reported to improved cathode performance. Also, instead of oxygen, other compounds such as nitrate, sulfate, iron, manganese, selenate, arsenate, urinate, fumarate, carbon dioxide, and hexavalent chromium can be used as terminal electron acceptors in biocathode (Stams et al. 2006; Sun et al. 2009). Anodes and cathodes of a MFC can be in separate chambers as described, which is called dual-chambered MFC (Fig. 8.5). On the other hand, a single chamber may contain both anode and cathode, which is known as single-chambered MFC (Chaturvedi and Verma 2016). However, according to recent studies, the double-chambered MFC faces higher mass transfer resistance due to

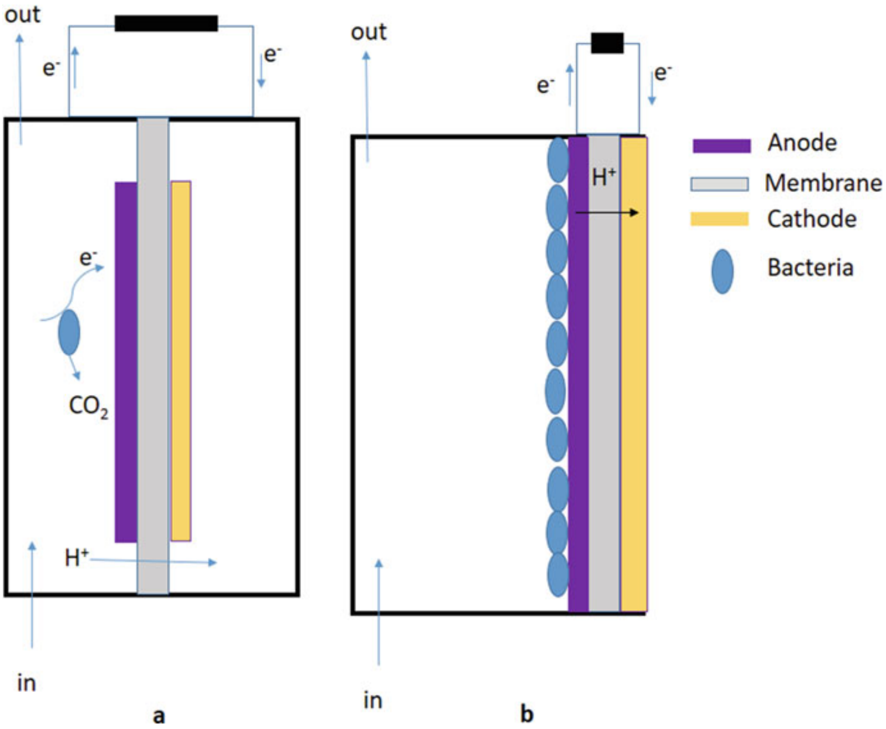

Fig. 8.5 (**a**) Double-chambered, (**b**) single-chambered microbial fuel cell in which substrates are in the anode chamber in (**a**), while (**b**) has only a single chamber for substrates or electrolytes. Bacterial biofilms are used in single-chambered apparatus on anode (Chaturvedi and Verma 2016)

the exchange membrane than the single-chambered MFC. So, double-chambered MFC showed a relatively lower power outcome than that of single-chambered MFC under the same operating conditions (Rajagopal et al. 2011).

However, the efficiency of converting chemical energy to bioenergy primarily depends on the substrate composition, and a wide range of feedstocks have been tried as fuel for MFC, especially artificial and real wastewaters (Schröder 2007). The substrate simultaneously influences the bacterial community in forming a biofilm on the anode as well as the power density and coulombic effect of the MFC performance (Chae et al. 2009). Although initially MFC employed simple low-molecular-weight substrate like glycose, fructose, acetate, etc., later complex carbon sources, especially wastes including animal manures, e.g., pig manure (Min et al. 2005), were in the main focus as fuels for MFC (Chaudhuri and Lovley 2003; Bond and Lovley 2005; Niessen et al. 2005). Similarly, different bacteria have been used for electricity generation in MFC, starting with *Saccharomyces cerevisiae* and bacteria such as *Escherichia coli* in 1911. Studies showed that the mixed culture or microbial consortia from natural sources perform better in MFC than laboratory-grown pure culture of bacteria (Ha et al. 2008). So, anaerobic reactor sludge or animal manure can preferably be used as fuel for MFC, as they contain various types of bacteria with electricity generating activity including bacteria that have not been characterized yet (Park and Zeikus 2003; Rabaey et al. 2003).

8.4 Benefits

8.4.1 Energy Potential

Considering the gradual depletion of fossil fuel-based energy sources, animal manure can surely be an emerging and valuable resource for renewable energy generation. Energy potential of manure depends on the type of animal, the composition of the manure, their feeding content, the bioconversion process, the form of energy produced used, etc. For example, chicken and turkey litter are assumed to a heating value of 4600 BTU/lb (Flora and Riahi-Nezhad 2006). Also, animal manure may be better fuel compared to feeds they consume, although manure composition, quality, and amount depend on feeds which is evident in case of cattle and buffalo manure. Cattle and buffalo graze on rice straw, but their manure is better fuel than original rice straw (Henuk and Dingle 2003; Cheeke 1999).

According to a study (Al-Hamamre et al. 2017), energy potential of animal manure was assumed as 18,453.25 TJ (5125.90 GWh) in Jordan generated from 4.8 million animals including poultry (92%), sheep (5.48%), and goats (2.02%). Another study reported annual energy potential from animal manure in Turkey would be 83.22 TWh (Ozcan et al. 2015). The annual electricity generation in Malaysia was found to be 8.27 TWh (Abdeshahian et al. 2016). However, the practice of bioenergy production from animal manure is still in the budding stage, and implementation of national and international policy with effective planning is required to increase the production of bioenergy decreasing our dependence on nonrenewable energy sources.

8.4.2 Safe Waste Disposal

The environmental impact of energy production from animal manure is far-reaching. While land spreading of animal manure is the most common practice for manure utilization, direct land spreading not only risks the spreading of potential pathogenic organisms to air or water bodies but also causes the emission of a large amount of NH_3, NO_x, and N_2O leading to terrestrial acidification, particulate matter formation along with eutrophication, and overall climate change (Billen et al. 2015). Moreover, co-digestion of animal manure and other wastes or plant biomass like water hyacinths provides an opportunity to control the pollution caused by such invasive plants along with harvesting energy (Lu et al. 2010). Besides, the livestock manure contains different harmful substances such as growth factor, antibiotics, and heavy metals, which can pollute the environment, especially water, and may get introduced into the food chain of humans leading to different diseases and disorders (Abdeshahian et al. 2016).

8.4.3 Reduction in GHG Emission

Unplanned or poorly managed animal dung is a major contaminant polluting air and water. If animal waste is not properly treated and disposed, it accounts for 18% of overall greenhouse gas emission, 65% of anthropogenic nitrous oxide, and 64% of anthropogenic ammonia emission (Steinfeld et al. 2006). Also, there is a link between N surplus and GHG emission. Each kg of N surplus leads to the GHG emission of about 30–70 kg of CO_2 equivalent (Oenema et al. 2006). A study estimated annual methane production from livestock/poultry manure to be 162,138 kg in 2016, while poultry, cow, and sheep produce 3,291,175, 326,200, and 305,750 ton/year, respectively (Safieddin Ardebili 2020).

So, biogas production reduces GHG emission, about 4 million tons of CO_2 equivalent/year in an effective way considering particularly CH_4 and NH_3 (Nielsen et al. 2002). So, while anaerobic digestion produces methane in the form of biogas to use as fuel or produce other energy forms with higher values, this application reduced the emission of CH_4 into the environment. It should be considered that CH_4 has 22 times greater greenhouse effect than CO_2 (Sheffield 2002a).

On the other hand, combustion of fossil fuel is the main contributor to GHG emission leading to global warming phenomenon. So, dependency on fossil fuel is one of the major obstacles to control GHG emission and to prevent global warming.

8.4.4 Energy Supply in High-Altitude Plane

High-altitude arid regions of the world have very harsh natural conditions such as extreme temperature, drought, etc. with scarce source of energy. The energetic resources available should be considered as renewable, even though the energy limitations lead to the limited use of energy as well only for preparing foods, heating households during the winter, etc. (Vankat et al. 2010). So, the main energy resource here is solar energy enabling plants and consequently other animals and humans to using the energy derived from solar energy (Vankat et al. 2010). Animal dungs are only source of draught power and fuel available and considered highly valuable (Vankat et al. 2010). Conventionally, manure is collected by girls and women, which is then dried and stored for use. For example, in a Himalayan village, Kargayak, yak and horse dung are collected from open fields, while sheep and goat dung are collected from stable. The manure collected is used to meet up the daily energy demand of the inhabitants by supplying 1411.15 kg per capita/year and 3.87 kg per capita/day for heating and 4466.21 MJ per capita/year and 12.24 MJ per capita/day for cooking (Vankat et al. 2010). Likewise, the high Altiplano region of the Andes Mountains can be characterized with dried and poor soil composition as well as adverse atmospheric conditions due to runoff and evaporation of the water (Winterhalder et al. 1974). They collect and use dung from llama, sheep, camelid, alpaca, and cattle along with kerosene, woods, or grass as fuels. Also, they reported use of mixture of both llama dung and cattle dung, although they did not use sheep manure as fuel due to acrid smoke produced while being burnt. More importantly, they prefer animal manure over wood because of ease of collection. So, animal manure should be considered as a reliable and satisfactory source of energy or bioenergy instead of woods or fossil fuels in high-altitude regions.

8.5 Challenges and Recommendations

Despite the huge energy potential of animal manure, bioenergy generation from this source has not been a common trend yet. Using manure as fuel itself creates some challenges. The first challenge is the high moisture content of manure (e.g., 5–40% in poultry litter, 66–97% in swine solids) along with low bulk and energy densities, differential chemical composition, and also a high ash content (Bolan et al. 2010; Zhu 2006; Stephenson et al. 1990). The low bulk density determines the distance manure can be transported economically for use as fuel. However, the chemical composition of manure with a high level of Cl, N, and S differs from conventional fuel and may cause problems in broiler or other downstream components including metal corrosion (Cl), interfering with the catalytic process of NOx and Sox removal (As) or in systems with no emission control leading to higher emission of NO_x, HCl, and SO_x (Ro et al. 2018). On the other hand, high ash content may cause slugging or fouling, making the cleaning process rigorous to maintain the stability of efficiency.

So, to use animal manure to produce energy in a coal-based plant, pretreatment of biomasses before using in the conversion process is required to achieve the coal-like properties.

Besides, the level of S and N in animal manure, especially poultry manure, is a matter of concern in case of the gasification plant. S is converted to corrosive H_2S which damages the heat exchange system of the plant, and the emission of N- and S-derived pollutant from these plants has a negative environmental effect (Virmond et al. 2012; Fernandez-Lopez et al. 2015). So, the development of technology or pretreatment of manure to reduce the level of S and N should be encouraged.

Along with the technological complications or limitations, lack of awareness, and lack of understanding, the technology is more common, especially in case of rural and underdeveloped regions of the world. So, they fail to establish replicable projects or to manage potential financial partners or governmental support. Likewise, a study demonstrated that the development of biogas or bioenergy industry depends more on policy incentives, rather than feedstock availability or technological advantage (Bangalore et al. 2016). So, international and national policymakers should be proactive to take the required steps to ensure green power generation from animal manure.

The financial barrier also needs to be considered to ensure energy coverage through waste to energy projects. Rural people and local institutions cannot afford the installation of these technologies at large scale without adequate financial support from the government or other organizations. Moreover, training on various aspects of renewable energy and its technologies are absent or not well designed as a part of the national curriculum at the university or the college level in most countries of the world (Surendra et al. 2011). Such a lack of technical knowledge has made the operation and maintenance of this technology more costly.

Lastly, political instability worldwide constrains the development of renewable energy. International and national politics can play a significant role in the development, while the frequent changes in the political system and changes in the governments affect the long-term planning and policy regarding the development of renewable energy from animal manure.

8.6 Conclusion

Considering the waste-to-energy concept, animal manure for energy production is undoubtedly a significant resource. While different approaches of energy conversion including gasification, pyrolysis, anaerobic digestion, microbial fuel cells, etc. are available, the conversion process and technology should be decided based on the purpose and utility of energy production, manure quality and availability, manure types, as well as infrastructure and economical considerations. However, despite all challenges and shortcomings of the available technologies, animal manure should be considered as one of the major sources of sustainable energy.

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Chapter 9
Animal Manure for Soil Fertility

Nadira Naznin Rakhi and Abdullah-Al-Jubayer

Abstract An exponential rise in world food demand pressurizes agricultural land to increase productivity to meet the demand. Overcultivation, removal of crop residues, and excess use of inorganic fertilizers leave agricultural lands exhausted and with poor nutrient contents. Although the use of mineral fertilizers had thrived in recent decades, their cost and environmental consequences had put their benefit in question. On the other hand, the use of animal manures including both livestock and poultry manure in agricultural lands has been practiced since ancient times. So, considering the current demand of crop productivity and the environmental impact of commercial fertilizers, using manure as fertilizers has been again in practice. Manure application, either fresh or compost, plays roles in improving soil physical and chemical properties directly or indirectly improving soil fertility through decreasing plant pathogens, increasing microbial interaction optimal for plant growth, etc. So, this chapter summarizes the manure application strategy and impacts regarding soil fertility.

Keywords Tillage · Composting of manure · Denitrification · Enteric pathogens · Soil health

9.1 Introduction

Soil fertility can be defined as the capability of the soil to provide an adequate amount of balanced nutrients for sustainable productivity with the maintenance of soil health and the environment (Karlen et al. 2008). However, the soil may not always be able to supply the adequate nutrients required for the proper growth of

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_9

crops and plants (De Meyer et al. 2011). On the other hand, soil erosion, faulty agricultural practice, and desertification wane down soil fertility and productivity (Eswaran et al. 2001). Repeated cultivation also causes a huge depletion in soil pH and Ca and Mg content (Liu et al. 2006). More importantly, the ever-increasing population has been creating a huge pressure on the agricultural food production demand, which in turn has been leading us to depend on fertilizer, either organic or inorganic. So, fertilizer is considered to be an important source of nutrients to improve soil health and productivity and also to ensure yield at maximum (Alimi et al. 2007).

From the middle of the nineteenth century, chemical fertilizers have been used by producers, as they were low-priced and widely available. Although it showed short-term benefits, it provoked severe side effects in the long-term scale such as erosion, compaction, and losing natural fertility along with the invasion of toxic chemicals in the food chain (Moral et al. 2005). The rise in the fossil fuel price also resulted in a hike in the price of inorganic fertilizers. Additionally, consumption of crops produced by using inorganic fertilizers is found to be responsible for different chronic diseases such as hypertension, cancer, and stroke (Udoh et al. 2005; Ademiluyi and Omotoso 2007). Some of these fertilizers contribute to toxicity by releasing heavy metal into the soil, which is uptaken by plants and in turn by animals (McLaughlin et al. 1996). Besides, liming agents and fertilizers produced from the waste of industry can be loaded with several heavy metals such as Pb, Cu, Cd, Zn, etc. These may accumulate in the soil due to the continuous use of those agents, which is a threat to the environment and soil health (Harrison and Webb 2001). So, despite the increase in the use of mineral fertilizers in recent decades, their cost and environmental consequences had put their benefit in question, and a suitable alternative to those chemical sources is necessary, while organic sources such as animal dung were found to be a potent one as a nutrient source (Fageria and Baligar 2005).

The use of animal manures in agricultural lands has been practiced since ancient times (Bayu et al. 2005). Animal manure is found to be favorable to soil fertility by enhancing drainage, aeration, water holding capability, and friability (De Silva and Cook 2003; Gbenou et al. 2017). Moreover, the bioavailability of gross minerals and toxics is also declined (Indoria and Poonia 2006). However, both livestock and poultry manures are used for such purpose, which contain high amounts of nitrogen, phosphorus, sulfur, calcium, magnesium, sodium, and potassium along with a trace amount of other useful inorganic matter such as iron, copper, zinc, etc. (Almeida et al. 2019). Also, waste from animal husbandry and poultry industries becomes a big burden if not properly disposed of. So, the use of animal manures as fertilizers can both minimize the cost of fertilization and provide a long-term benefit in terms of improving soil nutrient contents. The benefit of manure is largely determined by the composition of manures, which in turn depends on animals' diet, species, and the preparation process of the manure (Bayu et al. 2005). Commonly used animal manures come from cattle, swine, horses, and sheep. Along with the direct impacts of animal manures to improve soil health, manure increases soil organic content, serving as a source of energy and carbon to soil microorganisms, and prevents soil erosion (Bayu et al. 2005).

So, animal manure can not only increase productivity according to increasing demand in response to population growth but also restore the exosystemic balance, which was disrupted by traditional inorganic fertilizers.

9.2 Animal Manure as Fertilizer: Past, Present, and Future

European farmers used animal manure as fertilizer in 6000 BC (Bogaard et al. 2013). So, the practice of animal manure for soil health and crop production is not new at all. Manure has been an agricultural resource of great significance since the early years of agricultural development till the nineteenth century even in developed countries including the USA (Home 2006). According to early reports from the US Department of Agriculture (USDA), manure was considered to be so significant resource for farming that the neglect of this resource was highly discouraged. Also, the annual production of manure from cattle in the USA at that time was worth of one billion US dollars (Rayne and Aula 2020). According to those reports, the USDA also worked to raise awareness among the farmers not to replace manure completely with commercial fertilizers. Later, after the Second World War, livestock operations bloomed with the concomitant increase in the production of commercial fertilizers (Home 2006). But the decreasing land availability for agriculture and the increasing population led to today's practice of commercial inorganic fertilizer-dependent agriculture, which in turn necessitates the restoration of the quality of agricultural land primarily through the use of organic fertilizers. Likewise, the agricultural-dependent economy of several countries in Asia including India experienced environmental issues associated with pesticides and fertilizers, which has led to the rise of the demand for organic manure practice (SHUKLA 2014). Besides, African countries such as Ghana reported an increase in the corn yield from 55.6% in 1996 to 120% in 1998 due to the application of manure alone, proving its global implication and significance (Abunyewa et al. 2007). Also, a sharp rise in the production efficiency of agriculture has been reported from other countries after the replacement of commercial chemical fertilizer with animal dung (Risse et al. 2006). As a result, heightened public and consumers' interest in organic farming has contributed to the recent increase in the use of animal manure as fertilizer (Antonious 2016). Most commonly used manures in the USA are cattle and chicken manure, while other sources such as horse, sheep, goat, turkey, rabbit, etc. are widely used around the world (Faostat 2016). However, the amount of manure applied to fields depends on various factors, including the composition of the manure, the available nutrients of soil, the crop to be grown, and several environmental parameters (Eghball et al. 2002). According to an estimate by the USEPA, 900 million Mg of manure was produced from 2.2 billion livestock in 2007 (USEPA 2013), while in 2012, about 275,000 farms in the USA used animal manure as fertilizers, which represent about 8.9 million hectares of cropland (Hamer and Scuse 2010). However, a drastic increase in the global production of manure nitrogen (N) has been noticed from 1998 to 2014 (Zhang et al. 2017).

9.3 Nutrient Value of Animal Dung as Fertilizer

Unlike the commercial NPK fertilizers, animal manure is valued not only as a nutrient source of N (nitrogen), P (phosphorus), and K (potassium) but also as a provider of many secondary nutrients such as micronutrients, which are required by plants. But the exact nutrient value differs depending on animal type (Table 9.1), food provided, collecting process, storage, application method, and climate (Risse et al. 2006).

Also, inorganic and mineralizable P of manure have made up the available P. According to some research, this value in animal manure was found to be better or equal compared to the inorganic sources (May and Martin 1966; During and Weeda 1973), while the lower response was also noticed in few works (Goss and Stewart 1979). However, further research is necessary for the accurate estimation of available P value (Risse et al. 2006).

9.4 Manure Application Strategies

Land application of animal manure as fertilizer includes numerous strategies considering the different combinations of manure composition, crop system, soil type and composition, and farm management constraints (e.g., financial, personal, regulation, or others) (Meisinger and Jokela 2000). Raw manure in solid or liquid form as well as composted manure can be used as fertilizer. Solid manure usually uses the surface application strategy, while sometimes it gets subsequently incorporated. On the other hand, liquid manure can be applied following diverse strategies of surface spreading, such as from a tanker or by irrigation, or the combination of surface spreading and incorporation where manure is applied on the surface and tilled into the top soil layer, or shallow injection, or deep injection to a depth of >10 cm in the soil. Besides, the timing of manure application may be different. For example, many of those approaches can be applied before planting or during the crop season

Table 9.1 Nutrient scale of manure (Bates and Gagon 1981)

Type of dung	Nonliquid system (mg/kg)			Liquid system (kg/1000 L)		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O
Dairy	3–8	1–8	1–16	0.4–6.1	0.2–2.5	0.2–6.9
Beef	2–10	1–7	2–15	0.7–4.4	0.1–3.5	0.6–3.6
Swine	2–14	1–31	1–9	0.1–7.3	0.1–7.5	0.1–5.9
Poultry	2–66	1–48	1–28	4.2–9.0	1.6–10.9	1.6–4.7

However, nitrogen is very critical for plant growth, which can be derived from manure application, and N content in manure can be estimated by the following model (Sims 1986):
Available N = Fi Ni + Fm No
where:Ni and No = inorganic and organic N, respectivelyFi = the fraction (0–1) of inorganic N that is availableFm = the fraction (0–1) of organic N that is mineralizable or decomposable to inorganic N

between the rows of growing crops or even postharvest. However, tillage is not applicable for perennial plants considering the risk of root damage. Also, spreading manure on foliage is highly undesirable. So, liquid manure can be applied using trailing hoses to supply manure in narrow bands at the soil surface. Also, a coulter or shoe is often combined with trailing hose to maintain manure in a shallow trench. Besides, spreading into the air or sprinkler irrigation from lagoon effluent can also be applied in case of liquid manure (Sharpe and Harper 1997). However, application strategy may affect the outcome considering the yield of crops, physical and chemical characteristics of soil, the environmental effect of manure application, etc. While the effect on soil structure may be improved upon application of both raw manure and composts, the effects persisted for a longer period with a greater effect in case of compost application (Wortmann and Shapiro 2008).

9.5 Composting of Manure

Composting of manure is a globally recognized technique to reduce its weight and volume and improve the properties of manure through accelerating the natural decomposition for its use as soil amendment (Insam et al. 1996). Through this process, organic materials in manure are decomposed by the activity of microbes (bacteria, actinomycetes, and fungi) under controlled environmental conditions. Although composting procedure causes loss of some nitrogen considering volatile ammonia loss and nitrate loss through leaching, denitrification, etc., the net loss of N is much smaller than the direct land application of raw manure through rapid mineralization and leaching.

There are different procedures of composting, the most common of which are windrow, static piles, and in-vessel containers (Millner 2014). In either process, microbes degrade the manure, and such microbial activity significantly increases temperature ($\geq$55 °C). One beneficial effect of such high temperatures is that the temperature of the compost pile is high enough to kill the enteric pathogens in manure if the C/N ratio (20:1–40:1), moisture content (40–65%), and aeration level ($>$5% O_2 concentration) are maintained in the recommended range (Reynnells 2013). The US Environmental Protection Agency (EPA) and US Department of Agriculture (USDA) recommended maintaining the temperature of a compost pile at $>$55 °C for at least 3 consecutive days for static and aerated piles or at $>$55 °C for 15 days for windrow composting to ensure sufficient pathogen inactivation (Millner 2014; Reynnells 2013). However, high heat generated through microbial activity also degrades biodegradable solid organic matter into humus-like substances that make it easier to handle, store, and/or be used as fertilizer without adverse effects on the environment.

To improve the rate of composting and the quality of the final product, various modifications have been applied, including inoculation of microorganisms (Lopez et al. 2006; Sasaki et al. 2006). Factors associated with the effectiveness of composting include temperature, aeration, moisture content, pH, C/N ratio, particle size, degree of compaction, etc. (Lau et al. 1992; Stentiford 1996). The upper limit for moisture content of manure to be composted is recommended to be 65%, although manure with initial moisture content above 65% can be composted if the air supply is enough to maintain the growth and activities of microbes (Liao et al. 1993). Besides, dairy cattle slurry with 85.15% initial moisture content could be successfully composted on a laboratory scale (Genevini et al. 1996). However, bulking agents such as straw, sawdust, peat, peanut shells, rice hull, chicken litter, etc. can be used to improve composting efficiency. Co-composting of manure and the bulking agent provides balanced nutrients and moisture content for microorganisms to grow and convert the manure, because manure may remain too wet to be composted alone even after the separation of the solid fraction. For example, dairy manure has a high density and moisture content but a lower C/N ratio, which is exactly the opposite with rice straw. Ultimately, the mixture is the better feedstock for composting than either of the ingredients alone (Liu et al. 2011; Li et al. 2008). Also, co-composting offers odor-free process along with higher efficiency. However, sawdust has been considered as an ideal bulking agent, especially in case of composting pig manure. Because the structure of sawdust and its ability to absorb moisture form the ideal porous texture of compost pile providing optimal air and temperature for microbial activities (Bhamidimarri and Pandey 1996). Besides, sawdust has a high ammonia retention capacity, which helps to maintain the process odor-free.

Temperature and aeration are the most important parameters to be considered for composting. Although the maximum temperature of 55–65 °C can kill pathogens, the mesophilic range of temperature (45–55 °C) is ideal for biodegradation (Lau et al. 1992). But temperature exceeding 60–65 °C is not recommended as such high temperature would kill all microbes along with pathogens, causing the process to cease (Golueke and Diaz 1996). On the other hand, the air supply can be maintained by turning the pile regularly, by forcing aeration of the pile using pumps, by passive aeration, etc. Windrow composting usually implies mechanically turning compost piles at regular intervals, while forced aeration preferred in-vessel composting of manure. Interestingly, high aeration rate through continuous aeration has a cooling effect that severely affects the composting process (Lau et al. 1992; Liao et al. 1993).

Besides, degradation of lignocelluloses is a complex process requiring multiple enzymatic activities, and depending on the changes of lignin degradation ratio, composting process can be divided into three different phases. In the initial phase (0–15 days), the lignin degradation ratio increases from 0% to 4%; in the next stage of the fast degradation phase (16–30 days), the lignin degradation ratio reaches up to 28%; in the third and slow phase of composting, the lignin degradation ratio increases more up to 35% at the end of fermentation (Zhou et al. 2015).

However, while composting offers several advantages over conventional use, the environmental issues associated with composting, such as the emission of CH_4, NH_3, N_2O, etc., cannot be ignored. That is why nonconventional composting strategies including slow composting like vermicomposting have been proposed in some literature. However, the amendments of the process are ongoing to make composting more effective and ecofriendly.

9.6 Impacts of Fertilizer on Soil

9.6.1 On Chemical Properties of Soil

9.6.1.1 Soil Nutrient Status

Soil fertility can be measured by its nutrient status and its ability to provide nutrients endogenously or from external sources. Manure application increases the macro- and micronutrients of soil (Motavalli and Miles 2002), which can be translated as increased fertility. Manure application improves the soil chemical properties including pH, cation exchange capacity (CEC), organic matter, and organic carbon (C) and releases nutrients through mineralization. The ultimate nutrient status due to manure application depends primarily on the type of animal, feed intake of the animal, manure composition, storage and processing, etc. (Sution 1986; Antonious 2016).

The effects of manure application on soil chemical properties focusing on the nutrients and their availability are discussed below.

9.6.1.2 Soil Nitrogen Level

The global use of manure nitrogen (N) increased to 131 Tg of N per year (Zhang et al. 2017). The most common source was cattle manure, followed by goat and sheep manure, which together produced almost one-third of the global production in the past (Zhang et al. 2014), although poultry sources (chicken, duck, and turkey) have become a prominent supplier of manure N in recent years (Faostat 2016). However, contradictory results were found regarding the increase in the level of total N and nitrate upon the application of animal manure (Giola et al. 2012; Steiner et al. 2007). A study reported the gradual increase of N by up to 27% while applying a combination of inorganic fertilizer and dairy manure compared to inorganic fertilizer alone (Giola et al. 2012). However, ultimately, the effect on the level of N or any other nutrient depends on the mineralization rate, which is dependent on multiple factors including manure and soil composition, microbial activities, environmental factors, etc. (Eghball et al. 2002). Besides, according to Egball et al., the N released from manure is not readily available for plants, as the organic N needs to be converted first to inorganic form to be used (Eghball et al. 2002). However, another study supported the use of chicken manure with inorganic fertilizer to significantly increase the level of N content in the readily usable form for plants (Hou et al. 2012).

On the other hand, animal manure can reduce nitrate leaching, thus improving soil health significantly. The time of manure application and soil type majorly affect the rate of nitrate leaching from manure. For example, applying manure at a time when the plant does not absorb N, especially during high rainfall events, causes severe losses of nitrate with drainage water (Hou et al. 2012; van Es et al. 2006). Another study reported the lowest nitrate N concentration achieved with spring application followed by late fall compared to early fall application. But because of moisture, temperature, and other environmental factors, the leaching rate of nitrate N is often unpredictable, which leads to the overapplication of manure. But this practice causes serious harm to the environment. However, different studies reported variability in the rate of mineralization of N and nitrate, indicating the unpredictable nature of nutrient release from animal manure (Rayne and Aula 2020).

9.6.1.3 Phosphorus Level

Animal manure is rich in both organic and inorganic phosphorus (P), with inorganic orthophosphates accounting for 45%–90% of the P in manure, which are uptaken by plants (Bittman et al. 2004). However, N/P ratio in manure is much lower than the N/P ratio in plants, which causes the problem of excess P in soil. As a result, a large amount of manure needs to be applied to maintain the N supply required by plants, while excess P accumulates inevitably as the crops consume less P than N (Sharpley et al. 1993; Bittman et al. 2004). Application of dairy manure gradually increases the P level in the soil compared to no compost (Butler et al. 2008). Consequently, excess P along with N runoff leads to environmental hazards including eutrophication in water bodies. Also, the increased level of P from manure application leads to various soil characteristics including soil pH, organic matter content, and clay type (Chatterjee et al. 2014). However, the P content may vary extensively depending on the manure composition, storage and moisture content, feed of the animal, etc. (Fuentes et al. 2006). More importantly, it is plant-available P rather than total P that regulates the quality of manure as fertilizer (Barnett 1994). Extractable P forms in manure are available in the following decreasing order of plant availability: water soluble > bicarbonate-extractable P > sorbed P that is soluble in sodium hydroxide > acid-extractable P (Hou et al. 2012). Depending on the animal source, available P and total P may vary. For example, swine and poultry manure has high concentrations of total P, but dairy manure has higher content of water-soluble P. Such variation in the level of available P in manure is basically due to the differences of P forms provided in diets. As phytic acid in feed is the main form of P in cereal grains, swine and poultry manure contain high-level or phytic P than cattle due to decreased phytase production by those animals compared to cattle (Leytem et al. 2004; Fuentes et al. 2006; Bittman et al. 2004). However, unlike phosphate, phytic P remains as insoluble complexes in soil, making it less available to plant and leading to the loss through runoff (Leytem et al. 2004).

9.6.1.4 Trace Elements and Micronutrients

Trace elements are components needed in a concentration of less than 100 mg/kg on an average, among which the essential elements for the growth and the productivity of plants and animals are known as micronutrients (Alloway 2013). Manure application increases micronutrient availability significantly in the soil in case of extractable Cu, Zn, Mn, and B compared to control or inorganic fertilizer application

(Nikoli and Matsi 2011). Besides, liquid manure is thought to aid in the solubilization of metal micronutrients, leading to the formation of water-soluble complexes of organic compounds and metal micronutrients (Li and Shuman 1997; Matsi 2012). A study reported an elevated level of 60 different elements in soil upon the application of manure, although the accumulation of Zn was prominent (Sheppard and Sanipelli 2012). However, the regular manure application for a long time increases Cu and Zn level in the soil, while Zn level increases significantly especially in the topsoil after the application of 180 Mg/ha manure (Benke et al. 2008). Depending on the manure type, elevation of nutrient level may differ, as pig manure contains a significantly high concentration of Zn compared to cattle manure, pig dung, poultry dung, biogas manure, compost, and sewage sludge (Sager 2007). However, the level of nutrient or micronutrients in manure depends on the micronutrient level in the feed uptaken by the animal (Sheppard and Sanipelli 2012).

9.7 Soil pH

Soil pH shows an inconsistent relationship with manure application, while increasing pH was reported upon application of manure, such as rabbit, swine, goat, chicken, and cow manure (O'Hallorans et al. 1969; Ano and Ubochi 2007; Han et al. 2016). Calcium carbonate and bicarbonate in manure are responsible for pH changes in soil, as the addition of cations such as Ca and Mg and the presence of organic anions in manure can neutralize H^+ in soil (Eghball et al. 1996; L'Herroux et al. 1997).

The organic matter from manure improves the physical property of soil by forming stable aggregates and increases pH in acidic soil. In an experiment conducted by Adeniyan et al., two soil samples with increased acidity and severe nutrient deficiency were treated with organic manures or NPK fertilizer. The five animal manures used were cow dung, cassava peelings compost, poultry manure, rabbit droppings, and cane rat droppings. Cow dung showed the highest rise in pH levels, while NPK gave the lowest pH values. Overall, soil organic carbon, total nitrogen, available phosphorus, exchangeable potassium, and CEC values were comparatively better than NPK fertilizer in both soils (Adeniyan et al. 2011). In another study carried out in Omah, the outcome of organic manure and mineral fertilizer applied alone or in combination was investigated on tomato plants' growth. The results showed that higher doses of either organic manure or mineral fertilizers did not bring any significant positive outcome. However, when used together, there was a significant increase in yield and improvement of soil fertility for long-term use (Al-Wahaibi et al. 2014).

Animal manure containing a higher level of $CaCO_3$ may lead to increased soil pH to a greater extent compared to the manure containing lower concentration of $CaCO_3$, depending on the diet. As a result, poultry and pig manure can increase soil pH greater than cattle manure (Naramabuye and Haynes 2006). Contrarily, decreasing soil pH with the application of dairy manure was also noticed under irrigated and non-irrigated growing conditions (Hao and Chang 2002). A similar effect was found in the case of chicken manure (O'Hallorans et al. 1969). This phenomenon is explained as a function of nitrification and the simultaneous increase in electrical conductivity, as the increase in cation concentration replaces H^+ on the exchange sites (O'Hallorans et al. 1969). However, another study claimed that the changes in soil pH are not associated with manure application; rather the lime application on soil before the experiment showed the changes in soil pH (Ndayegamiye and Cote 1989). However, soil pH undoubtedly gets affected depending on manure type and soil conditions (Han et al. 2016)

9.8 Cation Exchange Capacity

In order to release essential nutrients to the soil, manure first needs to go through decomposition. The soil content then reacts with humus and water to enhance the cation exchange capacity of the soil (Adeniyan et al. 2011). Cation exchange capacity (CEC) can be measured by the retention of cations on the surface of the soil, which depends on the presence of organic materials and clay content in the soil (Goldberg et al. 2020). As manure contains organic matter, the application of manure changes the CEC state of the soil. Decomposition of manure along with plant materials causes an increase of negatively charged sites on carboxyl and phenolic groups, which causes an increase in the CEC (Muller 1933). The detail of the process is mentioned in the book *The Nature and Properties of Soils* written by Professor Nyle C. Brady (Daniels 2016). The pH of soil plays a crucial role in cation exchange capacity.

However, a study showed manure application for 1 year would not make any change in the CEC level, but the long-term application of manure for 13 years may increase CEC level proportionately, although this result was not consistent universally (Miller et al. 2016). On the other hand, irrigation showed an increased CEC level than unirrigated control land upon the application of the same amount of manure (Hao and Chang 2002). However, chicken manure increases CEC level to a greater extent compared to cattle manure, probably due to the higher C/N ratio in chicken manure than those in cattle manure (Miller et al. 2016).

9.9 Electrical Conductivity

The electrical conductivity (EC) of soil can be defined by its ability to conduct an electrical current. Manure application on soil showed a linear increase in the EC, primarily due to the availability of exchangeable K^+ along with various soil properties, such as organic matter and content, moisture content, salinity, and CEC (O'Hallorans et al. 1969; Grisso et al. 2009; Liebhardt 1976). The linearity of the relationship can be explained as the increased supply of nutrients and ions released from manure in the soil solution (do Carmo et al. 2016). A study by Miller et al. showed that 77 Mg ha^{-1} of stockpiled cattle manure with straw increased EC of the soil plot to 7 mmho/cm, while the unamended control plot had an EC of only 0.8 mmho/cm (Miller et al. 2017). The same study reported better performance with wood chips in combination with animal manure rather than straw bedding to maintain EC below the level inhibitory to the common crops (<4 mmho/cm). So, the type of manure and bedding in combination affects the outcome on EC of soil upon the application of manure.

9.10 Soil Organic Matter and Carbon

Depletion of soil organic matter (SOM) due to long-term cultivation is one of the biggest challenges in soil health and crop productivity, which can be amended by applying animal manure (Tiessen and Stewart 1983; Bakayoko et al. 2009). Application of chicken manure showed an increase of SOC by up to 1.95% compared to control treatment with no manure application, while cattle manure increased SOM, representing about 73% relative to the untreated control (Deryqe et al. 2016; Butler et al. 2008). However, the higher rates of applied compost did not make any difference in the SOM content of the soil.

Improved SOM in soil has an enormous impact on soil health,s including reducing erosion and runoff (Rhoton 2000), improved filtration (Franzluebbers 2002) and soil structure (Bot and Benites 2005), contribution to the C cycle, and even mitigating the effect of climatic changes (Lal 2004). However, the level of SOC depends on the manure composition and bedding types. For example, manure containing bedding rich in organic matter will increase SOC to a higher extent than manure with low organic matter containing bedding (Rayne and Aula 2020). Although conflicting pieces of literature are found on the relationship between manure and SOC, the World Bank reported 23, 109, and 267%, respectively, higher C sequestration rates in manure-amended soil from Africa, Asia, and Latin America compared to soils treated with inorganic fertilizers (The World Bank 2012). Also, 25% and 30% increases in SOC were reported while using pig manure alone and in combination with N, P, and K, respectively, from a 23-year-long study (Wang et al. 2015). Consequently, the same study showed that no nutrient application will cause the drop of SOC by 9% in the top 20 cm of soil. So, the SOC level is maintained or increased with the addition of fertilizers and animal manure compared to the initial level over multiple years. On the contrary, no effect of manure on the level of SOC was noticed in some studies (Ren et al. 2014). However, microorganisms decompose organic materials to harvest carbon as the source of energy, which releases nutrient in the soil. So, the addition of organic matters in soil causes an increase in microbial respiration, which may lead to decreased SOC, according to some studies (Marschner and Noble 2000).

9.11 On the Physical Properties of Soil

Manure application affects not only the chemical properties of soil but also the physical properties including soil water, structure, bulk density, resistance against erosion, etc.

9.11.1 Soil Water and Water Holding Capacity

Water scarcity is one of the largest challenges for agriculture, and the application of soil manure can be a way to amend this phenomenon, which is perhaps one of the most beneficial effects of using manure (Okono et al. 2013). The addition of manures to soil improves soil aggregation and pore size and volume to permit the storage of moisture. The characteristics are noticed even in sandy soils. The study shows improved water retention capacity by 19% and 27%, following 4 years of manure application at the rate of 9 ton/hectare (Bayu et al. 2005; Carter et al. 1992). Besides, a small increase in soil water increases organic C, although the organic C sequestration did not always respond to the presumed increase in available water content (Minasny and McBratney 2018). However, other studies showed a significant effect of organic matter on water retention (Ankenbauer and Loheide 2017; Ni et al. 2020). Organic matter in manure increases the adsorbing power of soil followed by improved water retention (Yang et al. 2014). Also, various other properties of the organic material itself may lead to increased water retention in soil. In the case of biochar-amended soil, field capacity, permanent wilting point, and available water content increased by 38, 58, and 14%, respectively, as pores in biochar also act as capillaries for increased water storage (Ni et al. 2020). Water holding capacity is the water in soil available for plants and is between the value of field capacity and permanent wilting point (Bauer 1974). However, poultry and cattle manure were found to cause higher water retention capacity in treated soil compared to untreated control soil or NPK fertilizer-treated soil (Ould Ahmed et al. 2010; Nyamangara et al. 2001). Moreover, partially decomposed horse-cow manure increased soil water content by 10.2% in even sandy loam compared to untreated soil (Bouyoucos 1939).

Another parameter called infiltration is used to define the process by which water enters the soil through the topsoil or soil surface by downward flow (Hillel 2003). Ponding or runoff occurs when the infiltration capacity of soil gets saturated (Assouline 2013). So, to prevent runoff and runoff-related problems such as eutrophication, manure application can be considered to increase infiltration capacity. Studies showed a reduction in the runoff by as much as 82% in soil with no tilling and as much as 42% in chisel-plowed soil (Yagüe et al. 2016). Manure improves infiltration capacity through soil aggregation probably, especially when the manure contains high concentrations of Mg and Ca ions (Chan and Heenan 1999).

9.11.2 Soil Temperature

Soil temperature and its fluctuations affect various activities of soil and the status of soil health including its biological activities, nutrient cycling, and nutrient uptake (Wallenstein et al. 2010). Organic compounds including manure affect soil temperature. Studies showed that applying poultry manure at the rate of 7.5 Mg/ha decreases the soil temperature by 2–2.3 °C (Agbede et al. 2008). Lowering the temperature through the application of manure is linked to improved water retention and the protection of soil against temperature fluctuations (Pinamonti 1998). However, the opposite report of increasing temperature upon compost application on soil has also been reported, which was probably caused by the reduced evaporation caused by compost application (Deguchi et al. 2009). It should be noted that evaporation has a cooling effect on the soil surface, so inhibiting evaporation would result in temperature increase naturally. However, improved soil structure and porosity as a result of manure application also decreases thermal conductivity of soil, thus forming insulation of organic matter to decrease soil temperature during the summer or to keep the ground warm during the winter, although the warming effect is negligible compared in snow-insulated soil where applicable (Zhu et al. 2019). This complex relation between the manure insulation and soil temperature was evaluated in several studies, among which Rees et al. reported a reduction in temperature by 0.32 °C in the case of manure application on potato hill with a 8% slope and an increase in temperature by 1.03 °C in the case of 11% slope (Rees et al. 2014). So, soil temperature may depend on the manure composition, the application time of the year, amount of manure, other physical properties of soil, etc.

9.11.3 Bulk Density

Bulk density of soil means the weight of dry soil per unit bulk volume of soil, which can be defined by the density of mineral particles, organic matter, and their packing arrangement (Bauer 1974; Rivenshield and Bassuk 2007). Considering the solid part and pore space of soil composition, the higher the pore space in soil relative to soil solids, the lower the bulk density for that soil (Rivenshield and Bassuk 2007). Chicken and cattle manure showed an opposite relationship between the rate of manure application and soil bulk density (Ahmad et al. 2016; Agbede et al. 2008). Manure decreases soil bulk density by increasing porosity. Also, applying organic matter like manure to soil causes the release of gums and polysaccharides by soil microbes, which increases soil aggregation and decreases bulk density (Dhaliwal et al. 2019).

9.12 Other Impacts

9.12.1 Animal Manure and Soil Biology

Soil microorganisms are the hallmark of soil health and a wide range of soil activities, while animal manure improves soil microbial community and also fungal diversity, leading to improved soil fertility and higher productivity (Ding et al. 2017). Manure application increases the growth of soil bacteria and stabilizes soil pH, contributing to a richer bacterial community (Cai et al. 2015). Thus, applying manure increases soil fertility by increasing the function of the microbial community which participates in nutrient cycling. Also, soil microbes contribute to the building of SOC, which is an important factor for soil fertility and productivity (Zhang et al. 2014). Mineralization of N and other nutrients by microbes also plays a role in nutrient enrichment of soil, leading to higher nutrient availability for plants and crops. This is evidenced by increased mycorrhizal colonization around the plant root upon manure treatment, which is especially significant in increasing the P uptake rate (Muthukumar and Udaiyan 2000; Kabir et al. 1997). Also, gram-negative bacteria are more abundant in the root region than gram-positive bacteria (Elo et al. 2000), while this ratio of gram-negative to gram-positive bacteria increases upon application of P-fertilized soil, which is directly associated with the organic matter present in soil (Kourtev et al. 2003). So, low fertility and extreme deficits in nutrient status of soil due to long-term cultivation, weathering, soil erosion, and leaching can be improved by supplying organic matter through manure application. Thus, manure application not only improves the microbial community structure but also makes it easier to absorb nutrients, especially the immobile one like P from soil (Zhong et al. 2010). Besides, considering the importance of soil pore and aggregates in nutrient exchange, fungal mycelia are of great significance to improve aggregate stability (Bolan 1991). Also, organic manure increases the ratio of macroaggregate formation through increased microbial activities and microbial biomass C, while cattle manure showed higher biomass C and associated microbial activities upon application (Parham et al. 2002).

On the other hand, inappropriate use of animal manure may reverse the positive impacts. For example, excess manure may lead to the P eutrophication and leaching, denitrification and volatilization of N, etc. (Daniel et al. 1998; Chambers et al. 2000). So, to get the beneficiary effects of manure application, appropriate dosage and strategies of manure application need to be implied.

9.12.2 Soil Productivity

Crop yield indicating soil productivity is an inevitable parameter of soil health, depending on soil type, climatic factors, plant genetics, soil and farming management, etc. Manure application increases grain yield and thus various yield parameters including harvest index, 100-grain weight, biomass, etc.

In a study conducted by Amiri and Fallahi (2009), the effect of deep litter cow and deep litter poultry manure on "Golden Delicious" apple growth was investigated. The manures increased nutrient content, organic matter, and cationic exchange capacity of the soil. The application of manures showed a positive effect on plants' nutrition status, improved yield, and increased fruit size (Amiri and Fallahi 2009). However, compared to untreated soil, studies showed that manure treatment resulted in 36% higher yield of dry shoot biomass by weight on a sandy clay loam and by 86% on a clay loam (Adeyemo et al. 2019). The same study showed an increase in 1000-grain weight and cob weight with manure application rate. On the contrary, another study reported a decline in total dry matter yield in case of maize after application of daily dairy manure compared to the NPK fertilizer (Khan et al. 2007). While this study found a decrease in 1000-grain weight of maize, no significant difference in grain yield was observed. This observation of no significant grain yield difference can be explained as the optimum availability of nutrients from the manure applied. However, another study reported decreased maize grain yield, which is likely because of the relatively lower concentrations of N, P, and K compared to the inorganic NPK fertilizer (Mahmood et al. 2017). Also, organic manure releases nutrients more slowly than inorganic NPK fertilizers. However, the outcome of grain yield depends not only on the manure composition or characteristic but also on the climatic and environmental factors (Taheri Rahimabadi et al. 2018). For example, liquid pig manure in below precipitation years does not affect wheat yield due to the low yield potential caused by the environmental factor. When precipitation increases above average, not only does grain yield increase by up to 50%, but straw yield also increases significantly (Nikièma et al. 2013). Similar results were found with chicken manure application (Faheem Jan et al. 2018). However, differences in grain yield may or may not occur, while no significant difference in gain weight was observed in those studies. On the other hand, higher rate of manure application can produce the same result in yield standard as the inorganic N fertilizer considering that the yield is dependent on the N available. Because N in synthetic fertilizer is more available compared to manure, manure releases N more slowly along with ammonia loss from surface application of manure (Jokela 1992).

9.13 Reducing Pesticide Dependence

Addition of animal manure to soil influences the structure and complexity of soil and soil food web, which in turn determines the status of nutrient cycling, plant disease, and soil parasite (Estevez et al. 1996; Haynes and Mokolobate 2001; Lalande et al. 2000; Lazarovits 2001). According to different studies, microbial biomass and activities increase with manure application, which leads to a shift in nutrient cycling. Such increased microbial activities were reported to increase bacterial population contributing to N cycle upon application of liquid swine manure (Lalande et al.

2000). Besides, increased nitrification with manure addition was also found compared to inorganic fertilizers (Kubát et al. 1999). Such contribution of manure in the food web and microbial community results in increased nutrient supply to plant and crop as well as may cause the problem of leaching, if the amount is not properly regulated. More importantly, with the increasing microbial biomass and diversity of bacterial species, plant disease rate is decreased through decreased competition between microbial consumers and increased predatory species, which in turn limits the growth of pathogens. Such mechanisms of disease suppression by manure application have been reported in the case of southern blight (*Sclerotium rolfsii*) in tomatoes upon swine manure application through increasing bacterial population antagonistic to *Sclerotia* (Bullock III and Risaino 1999). Besides, composted poultry manure was found to suppress the growth of *Phytophthora cinnamomi* probably through sustaining the biological activity of endospore-forming bacteria (Aryantha et al. 2000). However, other studies assumed that such suppression was due to the higher nitrogen content of pig and poultry manure by producing high ammonia and/nitrous acid concentrations in the soil (Lazarovits 2001), as fresh or composted cow, sheep, or horse manure did not show disease suppression. Interestingly, the addition of anhydrous ammonia and synthetic nitrite did not offer the same extent of disease control, while volatile fatty acids and acetic acid in swine manure were found effective against Verticillium wilt and potato scab (Lazarovits 2001).

Manure has effects on the nematode population as well. Nematodes are important in the agricultural system both as plant parasites and as a contributor to nutrient remobilization (Coleman et al. 1984). Poultry manure was found to significantly reduce parasitic nematodes, although such results were not observed in the case of cattle manure (Griffiths et al. 1994). Bacterivorus nematodes may get increased with manure application (Griffiths et al. 1994).

On the other hand, manure application may affect leaching and the degradation of pesticides directly. While the organic matter is well known to affect the activity, degradation, and persistence of pesticides (Benoit et al. 1996; Bollag et al. 1992), composted manure was reported to reduce volatilization of fungicides: methyl bromide and methyl isocyanate (Gan et al. 1998). Poultry litter was found to double the degradation rate of atrazine (Gupta and Baummer 1996). Such effects of animal manure are beneficial in decreasing leaching and associated eutrophication problem. Some studies reported better suitability and effect of compost compared to fresh manure considering the agronomic quality especially, as the stabilized organic matter in compost can act quickly through providing humus to the soil (Rynk 1994). Besides, composted manure showed better potential for reducing weed seed viability and thus reducing the demand for chemical herbicides (Edwards and Daniel 1992).

9.14 Reducing Runoff and Soil Loss

The agronomic carbon benefit of manure application includes the effects of manure on soil erodability. Improved infiltration, aggregation, and bulk density through manure application create a substantial impact on the runoff and soil loss from fields. The rates of runoff and erosion rates get affected by manure composition, characteristics, loading rates, time between application, and the first rainfall. Laboratory scale experiments showed a significant decrease in runoff and soil loss upon the application of cattle or poultry (Mitchell and Gunther 1976; Westerman et al. 1983). However, the long-term application of 55 years of beef cattle manure did not significantly influence inter-rill erosion in sandy loam soil (Gilley et al. 1999). Another study reported the effect of time on decreasing soil erosion by applying manure. It showed that cattle manure incubated for 15 days showed barely any effect on decreasing soil erosion, while cattle manure incubated for 30 days showed a significant decrease in erosion (Chandra and De 1982). This relationship can be explained as the minimum time requirement for organic matter in manure to influence the properties of soil and also highlights the limitations of short-term field trials in deciding the effect of manure on soil health. But the optimal time period for improving soil properties is still not known. On the other hand, the erodability value for manure is difficult to determine. Besides, many of the studies showed ambiguous results on runoff and erosion, although cattle or poultry manure was reported to decrease runoff and erosion in some studies. Also, most studies suggest less runoff and soil erosion after the land application of manure. The results using lagoon effluent or slurries are more difficult to conclude.

9.15 Limitations of Using Manure as Fertilizer

Even though animal manure has huge implications in agriculture and the environment, the need for extensive labor work for the collection, preparation, storage, and transportation strongly hampers their use. The bulk nature of manure makes it very difficult to transport to remote and distant places (Bayu et al. 2005). Compared to inorganic fertilizers, a large quantity of animal manure is required to ensure an equal quantity of inorganic elements. Furthermore, organic materials must be decomposed first before releasing essential nutrients to the soil. The slower rate of nutrient release may result in nutrient deficiency of soil. Inorganic fertilizers can provide a quick fix to the problem; however, high prices and high risk of soil acidification and groundwater contamination largely limit their sole use (Bayu et al. 2005; Rayne and Aula 2020). Furthermore, in rural areas where cooking fuel is scarce or expensive, animal manure is used as domestic fuel (Bayu et al. 2005). This creates a shortage of animal manure for use in arable lands as a fertilizer in the crop season. Overuse of manures may lead to the buildup of excess inorganic elements such as phosphorus, zinc, and copper, which if left untreated can lead to groundwater contamination (Jongbloed and Lenis 1998). It is very crucial to understand the need of the soil and plants grown on it and apply manures accordingly. Furthermore, the degree of decomposition should not be too long that it leaves the soil in poor nutrition status during the crop season.

9.16 Conclusion

Using animal manure as fertilizer is an ancient practice, although chemical fertilizers have been popular worldwide in recent decades. But considering the enormous and multidimensional impacts of manure on soil health, structure, and overall properties as well as the negative effects of chemical fertilizers on the environment and soil health, animal manure has grabbed attention for using as fertilizers. But considering the limitations of laboratory scale tests or field trials, more long-term studies are required for determining the optimal strategies and dosage for improved soil health and higher crop productivity.

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Chapter 10
Bioremediation of Used Motor Oil-Contaminated Soil Using Animal Dung as Stimulants

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Abstract The conventional method of disposing petroleum products in most developing countries is direct dumpingin into land and water (environment). The large portion of the global environment is contributed by the indiscriminate discharge of petroleum products. For many years, vehicle service centers polluted with used motor oil (UMO) have been neglected. The pollution of soil by used or virgin motor oil causes a detrimental effect on soil components and its microorganisms, agricultural productivity of soil and plant tissues, and human and other animal health. In view of the soil pollution caused by used motor oil, this chapter presents a study to describe the bioremediation potential and kinetic study of horse, elephant, and donkey dung and their combinations in treatment cells labeled T_2, T_3, T_4, and T_5 on UMO-polluted soil. Optimization of the biostimulation process using one-factor design of response surface methodology was also studied. The bioremediation potentials of the animal dung were evaluated for 42 days in the laboratory by evaluating oil and grease (O&G) content, bioremediation efficiency (BE), and total heterotrophic bacteria count (THBC) of the samples in the treatment cells. The first- and second-order kinetics fitted the oil biodegradation data. The kinetics showed that UMO-polluted soil treatment cells amended with T_2 (horse), T_3 (elephant), T_4 (donkey), and T_5 (combination of the tree) had lower half-lives and higher rate of biodegradation when compared with T_1 (unamended soil). The bioremediation efficiency and the kinetic modelling results showed that the animal dung (stimulants) bioremediation potential is in the order of $T_3 > T_2 > T_4 > T_5$. Also, based on 95% confidence level, the results of analysis of variance showed that both bioremediation time and biostimulant type affected O&G level removal. However, BE was only affected by bioremediation time. The cubic model developed for each of the responses was also found to be statistically significant with the probability value of less than 0.0001 and R-squared value greater than 90%. The multi-objective optimization carried out numerically revealed 42 days and E as best bioremediation

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_10

conditions to achieve maximum O&G removal of 85.4% at 51.7% BE. These maximum values of responses predicted were observed to be close to 83.73% and 48.01% obtained experimentally as maximum O&G removal and BE, respectively, under the same condition.

Thus, the study provides insight into the bioremediation potential of the animal dung and its applicability to develop an environmentally safe and robust treatment strategy for UMO-polluted soil.

Keywords Heterotrophic · Bacteria · Phytoremediation · Bioremediation · Bioaugmentation · Autochthonous microbes · Psychrophiles · Thermophiles

10.1 Introduction

Used motor oil (UMO) is known as brown-to-black oily liquid discharged from vehicle after the oil is changed (Abdulsalam et al. 2012). UMO is formed when unused motor oil is subjected to high pressure, mechanical strain, and temperature (Dorsey Jr et al. 1997; Abdulsalam et al. 2012; Adu et al. 2015). Chemical substances such as petroleum chlorinated biphenyls, hydrocarbons, chlorodibenzofurans, decomposition products, and lubricant additives are present in UMO (Dorsey Jr et al. 1997; Adu et al. 2015). Heavy metals from wearing of engine parts are also present in UMO (Keith and Telliard 1979; Dorsey Jr et al. 1997; Abdulsalam et al. 2012; Adu et al. 2015).

In oil-producing and industrialized countries, spilling of petroleum products in soil is a widespread environmental problem in many countries, particularly in African and Asian continents (Agamuthu et al. 2010; Stephen and Ijah 2011). The issue is severe in developing countries with poor regulatory policies (Agamuthu et al. 2010; Onuoha et al. 2011; Stephen and Ijah 2011; Ebenezer 2013).

For many years, spilling of oil at vehicle service centers has been neglected in many countries, and continuous oil accumulation causes serious environmental problem because of its associated hazard (Abdulsalam et al. 2012). The release of persistent, toxic, and bioaccumulative chemicals (polycyclic aromatic hydrocarbon, toluene, benzene, ethylbenzene, and xylene) caused severe environmental and health hazards. These pollutants find their ways into plant tissues, human beings, and animals by the movement of toxic substances in the environment (Ebenezer 2013). Pollution of soil with UMO and unused motor poses detrimental effect on soil components, plant tissues and its microorganisms, and animal and human health (Stephen and Ijah 2011; Adams et al. 2015; Adu et al. 2015). Excess spillage of the oil causes fire hazards, which leads to loss of lives and properties (Adams et al. 2015).

There are many ways of remediating contaminated soil, which include both physicochemical and biological methods. The latter is more efficient and economical than the former (Thapa et al. 2012). Despite the importance of technologies available for physicochemical method of remediating contaminated soil, they are hindered by some limitations. These limitations are (10.1) it destroys soil characteristics and texture, (10.2) it makes the environment toxic, (10.3) large-scale application is expensive, (4) it involves advanced technology, and (5) pollutants are not completely neutralized (Abdulsalam et al. 2012). Bioremediation is a biological treatment that offers an economical and promising remediation approach for contaminated soils. The process involved the biodegradation of pollutants by nonindigenous and indigenous organisms like fungi and bacteria or plants to detoxify, destabilize, or destroy pollutants or make them harmless since the pollutants are utilized as the carbon and energy source of the microbes (Ebenezer 2013; Sharma et al. 2014).

The specificity of the degradation process is the genetic potential of the specific microbe to add oxygen molecule to hydrocarbon and produce intermediates, which subsequently enter the cell's general energy-yielding metabolic pathway (Millioli et al. 2009; Thapa et al. 2012). Microorganisms such as bacteria are movable, and they respond chemotactically to contaminants by going toward it, while others like fungi form filament around the pollutants (Kumar et al. 2011; Thapa et al. 2012).

The indiscriminate discharge of used motor oil into soil and groundwater has become a significant problem today. The primary global concern today is the contamination of soil through oil spills, and it has caused grave danger to human health (Thapa et al. 2012). Soil contamination caused by an oil spill also causes the following: (1) groundwater pollution and decreased usage, (2) environmental issues, (3) resource loss, and (4) decrease in soil agricultural productivity (Thapa et al. 2012).

Given the high health risks, loss of biodiversity, and risks of further contamination posed by excessive discharge of UMO in the soil, this work aimed at evaluating and comparing the bioremediation potential of animal manure (horse, elephant, donkey, and mixture of the three dungs) as stimulants. The following were conducted to achieve the aforementioned aim of the study: determination of the physicochemical properties (oil and grease content (O&G), moisture content, pH, carbon, nitrogen, and phosphorus) of the UMO-contaminated soil, horse, elephant, and donkey dung, determination of bacteria count in the contaminated soil, conducting microbial analysis of the treatment in the treatment cells (total heterotrophic bacteria count) for a period of 6 weeks, and determination of the kinetic model and half-lives of the UMO biodegradation process in the treatment cells as well as the optimization of the bioremediation process.

In the last decade, tremendous efforts have been made toward reducing the pollutants in the environment using natural processes for treatment (Abdulsalam et al. 2012), but the use of elephant, horse, and donkey dung as biostimulants has not been reported. This dung was considered for this work since they are readily available at little or no cost and their usage as biostimulants can reduce environmental pollution, which occurs from their disposal without proper management.

10.1.1 Principles of Bioremediation

Remediation technologies offer developing countries (in tropical regions) a great advantage as far as biological methods are concerned, with high temperatures and humidity that favor biological reactions that can be used for phytoremediation and bioremediation (Uygur et al. 2010). Vidali (2001) described bioremediation as the utilization of living organisms, mainly microorganisms, to degrade the environmental contaminants into less toxic substances. Furthermore, bioremediation technology utilizes microorganisms to reduce, contain, eliminate, and/or transform the contaminants present in water, soils, and sediments into less harmful forms (Adams et al. 2015).

The two main approaches of bioremediation are bioaugmentation and biostimulation (Vidali 2001).

10.1.2 Bioaugmentation

Bioaugmentation can be defined as the introduction of a group of indigenous microorganisms or genetically engineered microbial strains to remediate the contaminated environment. It works well when there are no autochthonous microorganisms in the polluted soil, or the microorganisms do not have the metabolic capability to treat the contaminated soil (Thapa et al. 2012). To observe whether isolated microorganisms can compete with the autochthonous microorganisms, Venosa and Zhu (2003) tested ten different commercial isolated bacteria using weathered Alaskan crude oil in shake flask treatment cells. It was observed that the seeded microorganisms poorly competed with the indigenous microorganisms. Lee et al. (2011) also studied the microbial degradation of soil contaminated with Tapis crude oil by *Acinetobacter baumannii* T30C. Based on their findings, it was concluded that the application of nutrients was essential for enhancing bacteria growth and degradation activity, except in situations where the identifiable indigenous hydrocarbon-degrading microorganisms were too small and the introduction of biodegrading microbial strains became necessary.

Furthermore, Abdulsalam et al. (2012) obtained 60% total petroleum hydrocarbon (TPH) degradation in the soil contaminated with used motor oil using exogenous bacteria. Abdulsalam et al. (2011) achieved 66% of TPH degradation in the soil contaminated with used motor oil using a consortium of bacteria. Agarry and Oghenejoboh (2015) revealed that bioaugmentation exhibited 77.5% naphthalene degradation in the soil contaminated with 4% (*w/w*) naphthalene. Esedafe et al. (2015) showed 97.90% degradation of the polycyclic aromatic hydrocarbons in the refinery effluent after 360 h in mixed bacterial culture followed by 96% with *Pseudomonas* sp. (SB) and 93.40% with *Achromobacter xylosoxidans* (SB1). According to Sharma et al. (2014), the results obtained from bioaugmentation of

P. aeruginosa in diesel contaminated soil were observed to be 66% diesel oil degradation in 30 days incubation period.

10.1.3 Biostimulation

Biostimulation can be defined as the modification of the environment by the addition of nutrients, such as nitrogen, oxygen, phosphorus, and other electron acceptor species, to enhance the metabolic capability of the indigenous microorganisms and improve the rate of biological degradation of contaminants. If the natural microbial population in the site has the potential to degrade the contaminants but lacks the required nutrients such as nitrogen, oxygen, phosphorus, etc. to degrade them, it is important to use biostimulation. To induce the degradative activity of the microbial community, the missing components are added (Artin 2010). Most bioremediation systems employ some form of biostimulants, which are organic and inorganic nutrients.

10.1.4 Biostimulation Using Organic Nutrients

Many researchers have worked on bioremediation of contaminated soil using organic nutrient, some of the work carried out by other researchers are as follows.

In a finding by Abioye et al. (2012), on soil contaminated with 15% and 5% used lubricating oil and amended with three different biostimulants, namely, spent mushroom compost (SMC), brewers' spent grain (BSG), and banana skin (BS), it was concluded that the least (55%) oil degradation was achieved with BSG at 15% contaminant level compared to 92% oil degradation in soil contaminated with 5% oil when amended with BS. The difference in the percentage degradation was a result of the difference in the contamination level. Stanley (2013) studied the biodegradation of hydrocarbon in the soil for 6 months. He showed that the reduction levels of the TPH in the soil contaminated with 0.5% used motor oil were 97.8%, 92.6%, 81.6%, and 79.2% for amended soil goat dung (GD), poultry manure (PM), cow dung (CD), and unamended control soil, respectively. However, soil contaminated with 2.5% used motor oil has a level of reduction of TPH to be 54.4%, 60.5%, 54.1%, and 50.4% in amended goat dung (GD), poultry manure (PM), cow dung (CD), and unamended soil, respectively.

Similarly, Sarang et al. (2013), in their research, evaluated the effectiveness of cow dung, goat manure, and spent fruit residues as stimulating agents for the bioremediation of simulated fresh motor oil (10%)-contaminated soil and used motor oil-contaminated soil. They showed that cow dung has the highest biodegradation of fresh motor oil in the simulated fresh motor oil-contaminated soil with percentage removal of 73.47%, while spent fruit residue has the highest biodegradation of 74.91% used motor oil-contaminated soil. Agarry and Latinwo (2015) conducted a study to investigate and evaluate the effects of bioventing, attenuation, and brewery waste effluent amendment as biostimulation-bioaugmentation agent on biodegradation of diesel oil in soil contaminated with 10% (w/w) diesel oil. The result showed that after 28 days, 78.7% of the oil was removed from the soil amended with brewery waste effluent and 91.6% oil removal from the soil amended with a combination of bioventing and brewery waste effluent.

Furthermore, Abioye et al. (2012) showed that the soil contaminated with used motor oil (10% w/w) amended with brewery spent grain gave the highest percentage reduction of used motor oil (95%), followed by soil amended with banana skin (93%) and 92% reduction for the spent mushroom compost-amended soil. In another work by Orji et al. (2012), they reported that after 70 days, there was 62.96% total petroleum reduction when cow dung was used as a stimulating agent when compared with the control.

10.1.5 Biostimulation Using Inorganic Nutrients

The inorganic fertilizers have also been utilized as biostimulants throughout the world. Chorom et al. (2010) studied the effectiveness of inorganic fertilizer (NPK) as biostimulant for petroleum hydrocarbon degradation in the soil. They obtained percentage reduction of normal paraffin and isoprenoid in the range of 40–60% in less than 10 weeks. Agarry and Ogunleye (2012) conducted an enhanced bioremediation of soil contaminated with used motor oil using inorganic NPK fertilizer and non-ionic surfactant as independent biostimulation variable; after 42 days, they observed that there was 67.20% reduction in TPH concentration. Similarly, Abdulsalam et al. (2012) reported that after 70 days, 75% biodegradation of TPH was achieved in the biodegradation of soil contaminated with used motor oil in anaerobic fixed-bed bioreactor using NPK (20:10:10) and KH_2PO_4 as biostimulants. In another research by Abdulsalam and Omale (2009), after 42 days, they showed that there was 69.2% TPH removal in the bioremediation of used motor oil-contaminated soil using NPK fertilizer as inorganic biostimulant.

Also, Thangarajan et al. (2011) showed that there was 68.8% reduction in TPH in the bioremediation of hydrocarbon contaminated soil pile that was meant for landfill disposal after 56 days. Agarry and Oghenejoboh (2015) revealed that biostimulation (NPK fertilizer) exhibited 69.5% naphthalene degradation; Agarry and Owabor (2012) showed that 75.06% TPH degradation was achieved in the bioremediation of soil artificially contaminated with kerosene using NPK fertilizer as a stimulating agent. Udochukwu et al. (2014) showed that crude oil degradation in NPK soil sample was 68.5% after 28 days.

10.1.6 Biological Treatment/Metabolic Path

The biological degradation of contaminated soil occurs basically in two ways, which are aerobic and anaerobic.

10.1.7 Aerobic Degradation

Aerobic degradation is a situation whereby the microbes feed on the organic pollutant in the presence of an electron acceptor, such as oxygen, to produce water (H_2O) and carbon dioxide (CO_2) with the release of energy and more microbes. The molecular oxygen (oxidizing agent) is reduced, while the organic pollutant (reducing agent) is oxidized. The microbes (biomass) use the organic pollutants as their energy and carbon source, and hence the biomass quickly adapts and multiplies (Ebenezer 2013). The aerobic hydrocarbon degradation is shown in Eq. (10.1), while Fig. 10.1 shows the mechanism of aerobic hydrocarbon degradation. The organic pollutant is initially attacked intracellularly in an oxidative process, whereby the oxygen is incorporated and activated in an enzymatic reaction catalyzed by peroxidases and oxygenases. These degradation mechanisms convert organic pollutants step by step into intermediates of the tricarboxylic acid cycle (TCA, that is, C_4, C_5, and C_6). Biosynthesis of cell biomass takes place from the central precursor metabolites including acetyl-CoA, pyruvate, and succinate. Sugars that are needed for various biosyntheses and growth are synthesized by gluconeogenesis.

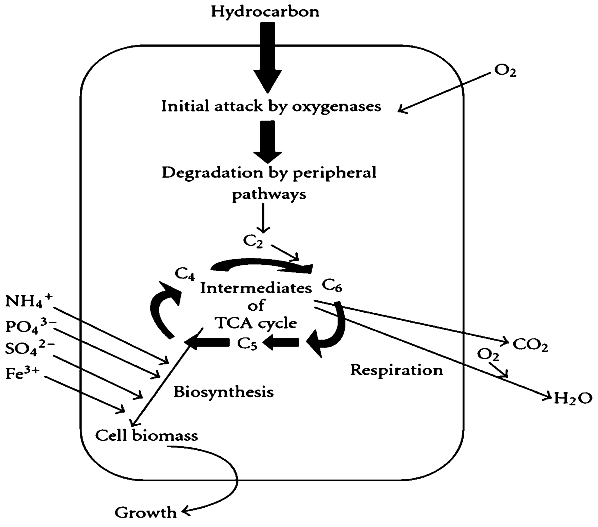

Fig. 10.1 The main principle of aerobic degradation of hydrocarbons by microorganisms (Das and Chandran 2011)

$$\text{Biomass} + \text{Organic pollutant} + O_2 \rightarrow \text{Biomass} + CO_2 + H_2O \qquad (10.1)$$

10.1.8 Anaerobic Degradation

Anaerobic degradation occurs in an environment under anoxic conditions (in the absence of oxygen); the microbes will utilize nitrate, sulfate, and bicarbonate as an electron acceptor (Ebenezer 2013). The anaerobic hydrocarbon degradation is given by Eq. (10.2).

$$C_7H_8 + 6NO_3^- \rightarrow 7CO_2 + 3N_2 + 4H_2O \qquad (10.2)$$

10.1.9 Biodegradability of Petroleum Hydrocarbon

In general, the degradation of water-soluble compounds is faster compared to compounds that are less soluble in water because bacteria in the unsaturated soil mainly occur in the interstitial water of the soil; hence, hydrocarbons in soil correlate to their water solubility (Jain et al. 2011). The composition of oil as a pollutant influenced biodegradation ability. For instance, kerosene (comprises almost exclusively medium chain alkanes) undergo complete biodegradation. In the same way, crude oil also undergoes biodegradation quantitatively; the degradation of petroleum has been reported to be increased when there are many species of bacteria compared to when there is only one species (Ebenezer 2013).

The normal alkanes, aromatic and normal alkylaromatic compounds in the C_5 to the C_{22} range, are usually biodegradable. The compounds are significant fractions of gasoline, diesel, and fuel oil. Low water solubilities of the normal alkanes, normal alkylaromatics, and aromatic compounds above C_{22} resulted in low rates of degradation. These compounds are common in heavier oils. The rates of biodegradation of fused or condensed aromatic and cycloparaffinic compounds with four or more rings are very low. These include most polycyclic aromatic hydrocarbon compounds (PAHs). The rate at which benzene, ethylbenzene, toluene, and xylene (BTEX compounds) are removed by aerobic metabolism is the same. The aromatic compounds are first oxidized to organic acids or phenols and then changed to long-chain fatty acids that are volatile and later breakdown to carbon dioxide and methane during anaerobic breakdown (Ebenezer 2013).

The anaerobic rates of degradation and biodegradation ability of compound varied significantly. Many chlorinated hydrocarbons are also readily biodegradable via aerobic and anaerobic breakdown. However, the use of bioattenuation should be considered carefully at high concentrations of chlorinated hydrocarbon compounds, due to the potential of producing higher toxic metabolites than the original contaminants. The knowledge of the relevant microbial metabolic pathways is essential. For example, vinyl chloride is produced as a metabolite of the anaerobic breakdown of TCE, which is highly more toxic than the precursor. As such, naturally occurring biodegradation would not be acceptable (Ebenezer 2013).

10.1.10 Factors Affecting Bioremediation

In order to optimize the conditions needed for biodegradation of polluted environment, the knowledge of the biodegradation triangle (Fig. 10.2) is needed, which consists of three factors, viz, the presence of organic pollutants as source of food and energy to the microbial community, the presence of microbial community that can degrade the pollutants, and the environmental condition (soil type, temperature, oxygen or other electron acceptors, nutrients, and pH) (Suthersan 1999).

10.1.11 Microbial Community for Bioremediation Processes

Microorganisms can be isolated from various environments to enhance biodegradation of organic pollutants present in the contaminated site, due to their adaptation to different environmental conditions. Microorganisms produce substances (such as enzymes and surfactants) which attack the contaminants. The degradation of

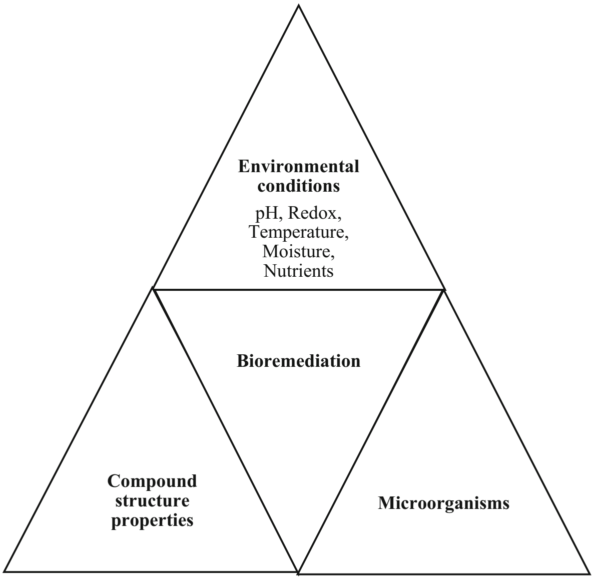

Fig. 10.2 Biodegradation triangle depicting factors required for bioremediation (Suthersan 1999)

petroleum depends on the population of the microorganisms available to degrade the oil through step-by-step process by which the degradation takes place (Ebenezer 2013).

Although the contaminated soil already has microbes, the amount required for effective bioremediation may not be available, necessitating the introduction of engineered microbes to augment the deficiency (bioaugmentation) or adjusting the environmental conditions and adding nutrients to enhance the multiplication of indigenous microorganisms (biostimulation) (Abdulsalam et al. 2013).

10.1.12 Environmental Factors

The growth and metabolic activities of the microbes capable of degrading pollutants present in the contaminated environment are influenced by the physical, chemical, and biological factors. These factors have a strong impact on the rate of microbial transformations, metabolic pathway, microbial density, and persistence of contaminants. In reality, microorganisms may be exposed to different conditions, including lack of essential growth factors, insufficient amount of inorganic nutrients, high pollution levels that stressed the population of the microorganisms, extreme temperature, and pH. The growth of microorganisms is slowed when the pollution level is very high, and metabolic activities are also inhibited, which may also cause loss of viability (Suthersan 1999).

10.1.13 Redox Potential

The reduction-oxidation (redox) potential (Eh) is related to the amount of oxygen in the liquid and gas phase. The oxygen concentration affects exchange rate with the atmosphere and respiration rate by soil microorganisms and plant roots. Respiration may reduce oxygen concentration, thereby lowering the redox potential and creating anaerobic conditions. These conditions can lower the rate of aerobic reactions and promote anaerobic processes such as denitrification, sulfate reduction, and fermentation. Eh of well-aerated soils can be in the range of 0.8–0.4 V and that of moderately reduced soils is between 0.4 and 0.1 V. Also, Eh of reduced soils measures about 0.1−0.1 V and that of highly reduced soils can be in the range of 0.1−0.3 V. Redox potentials are difficult to measure in the soil or groundwater and are not widely used in the field (Artin 2010).

10.1.14 Soil Texture

Soil permeability, moisture content, bulk density, and particle density are affected by the structure of the soil, which controls the way nutrients and oxygen are distributed. It is imperative to study the soil structure because sticky soil (clay soil) tends to be difficult to aerate, which then leads to low oxygen concentrations. This type of soil is not good for in situ bioremediation (Abdulsalam et al. 2013; Ebenezer 2013).

10.1.15 Soil Moisture Content

Since microbes reside in the film around the soil particles, soil moisture content is a major factor of biodegradation of pollutants in contaminated soil (Jain et al. 2011). This moisture serves as a medium through which organic components and nutrients are absorbed by microorganisms and wastes which are expelled from metabolic processes (Ebenezer 2013). Very high moisture content of the soil reduces oxygen penetration into the soil and hence impedes the growth efficiency and impacts the kinds of microorganisms that reside in the soil (Artin 2010).

The water content required for bioremediation of contaminated soil should be between 20 and 80% for landfarming. In other remediation techniques where there is no extra source of continuous circulation of air, 20% moisture may be adequate, while 80% moisture may be adequate when there is constant flow of air—such as bioventing, etc. (Artin 2010).

10.1.16 Temperature

In the biodegradation of pollutants, the temperature is considered as an important factor since it affects population and physiology of microorganism as well as the chemistry of the organic pollutants. At a low temperature, volatilization of the pollutants' light components is reduced since the oil becomes more viscous, thereby delaying the onset of bioremediation. The biodegradation of organic pollutants usually occurs between the temperature range of 30 and 40 °C in soil (Jain et al. 2011). The rate of biochemical reaction and microbial metabolism is affected by an increase in temperature; for every 10 °C rise in temperature, the reaction rate doubles. However, biomass begins to die as a result of decline in their activities at a temperature beyond the optimum, which can be explained in terms of categories of microbes based on optimal temperature range (Suthersan 1999; Vidali 2001). The three categories of microbes are as follows:

1. Psychrophiles: these are the organisms that have optimum temperature range of 0–15 °C, and they die when they are exposed to room temperature. However, facultative psychrophiles can grow at optimum temperature range of 25–30 °C.

2. Mesophiles: these are the organisms with optimum temperature range of 25–40 °C and mostly the inhabitants of the subsurface region. They are commonly found effective for bioremediation purpose.
3. Thermophiles: these are the organisms with optimum temperature of above 45 °C, such as thermophilic methanogens that prefer a temperature range of 55–60 °C.

10.1.17 Soil pH

Soil pH is an important parameter to be considered for microbial metabolism. Basically, pH is an indicator of hydrogen ion activity in the soil, and soil pH in the range of 5–9 is generally acceptable for biodegradation. pH of 6.5–8.5 is mostly considered to be appropriate for optimal biodegradation efficiency. Mineral soils are neutral to alkaline in conditions, while organic soils in wetlands are often acidic. Most heterotrophic bacteria and fungi are favored by neutral pH, while fungi are more tolerant in acidic medium. Studies have shown that degradation of oil increases with increase in pH and that optimum degradation occurs under slightly alkaline conditions (Venosa and Zhu 2003). For highly acidic soil, soil pH can possibly be buffered by adding crushed limestone or agricultural lime (Vidali 2001). Most heterotrophic bacteria are favored at pH of 7.0, while extreme soil pH would have a negative impact on the ability of microbial populations to degrade hydrocarbons (Jain et al. 2011).

10.1.18 Nutrients

Carbon makes up a large portion of the total protoplasmic material of a microbial cell. Carbohydrates, amino acids, proteins, vitamins, purines, pyrimidines, nucleic acids, and other substances constitute the cell mass. Apart from carbon, cell material is mainly composed of various elements such as nitrogen, hydrogen, and oxygen. These four chemical elements make up about 95% by weight of living cells, and two other elements including calcium and phosphorus constitute about 70% of the remainder (Suthersan 1999).

10.1.19 Techniques of Bioremediation

In the remediation of contaminated soil, there exist various techniques. The type of soil, quantity and types of pollutants, treatment costs, and conditions of the environment are the factors considered when choosing the best treatment techniques (Jain et al. 2011). The treatment techniques are in situ and ex situ.

10.1.20 In situ Techniques of Remediation

In situ remediation involves the treatment of soil at the contaminated site without excavating. This technique is applied when the polluted area is very wide since excavating the soil will be very expensive and ranges from partially closed systems to the ones that are completely open (Artin 2010; Jain et al. 2011). The in situ technique of bioremediation includes bioventing, phytoremediation, biosparging, and land treatment.

10.1.21 Land Treatment

This is a comprehensive technology of bioremediation in which the polluted environment is aerated periodically to aid the interaction of the pollutant at the contaminated site with oxygen. The only advantage of this technology is that it requires convectional farming equipment (Jain et al. 2011).

10.1.22 Bioventing

Bioventing is a technique in which vacuum is applied from the extraction well bored close to the contamination point above the water table. The contaminants are biodegraded by interacting with the oxygenated air. The evolvement of carbon dioxide in the well shows biodegradation activity. This technology is cheaper when compared with when hydrogen peroxide or nitrates are utilized as electron acceptor source (Jain et al. 2011).

10.1.23 Biosparging

Biosparging is the process of increasing the concentration of oxygen in groundwater by injecting pressurized air below water table to enhance the rate at which pollutants are degraded by indigenous bacteria. Saturated zone mixing is increased by biosparging, which ensures groundwater and soil contact. The flexibility in the system design and construction is because of the low cost and ease of small-diameter injection point installation (Vidali 2001).

10.1.24 Phytoremediation

Huge success has been recorded when technology involves the use of microbes to degrade petroleum-based pollutants; however, success is limited when it involves different types of metals and residual organic pollutants (Vidali 2001). The use of plant for remediating environment polluted with metals and organic pollutants transforms, immobilizes, and accumulates the pollutants by acting as filter (Schnoor et al. 1995; Tangahu et al. 2011; Sophia and Shetty Kodialbail 2020; Yan et al. 2020). Phytoremediation is a technology involving the extraction or removal of pollutants from water and soil by plants. The term was first used in 1991 (Schnoor et al. 1995; Vidali 2001; Yan et al. 2020). This technique is of five different types, namely, phytoextraction, phytotransformation, phytostabilization, phytodegradation, and rhizofiltration (Vidali 2001).

10.1.25 Ex situ Techniques of Remediation

The time required for ex situ techniques is generally shorter than that of in situ techniques because they can easily be screened, homogenized, and monitored, which gives more uniformity of treatment. Contrary to the in situ technology, it requires excavation of the soil, which leads to increase cost of treatment. This technology includes composting, landfarming, biopiles, and bioreactors (Jain et al. 2011).

10.1.26 Composting

Composting is the process of combining nonhazardous organic stimulants (agricultural waste and animal manure) with oil-contaminated soil. The organic stimulants ensure the formation of rich microbial community whose activities lead to heat generation (Jain et al. 2011; Kumar et al. 2011).

10.1.27 Landfarming

This involves the periodic tilling of excavated polluted soil spread on a prepared bed with the aim of stimulating indigenous biodegradative microbes and accelerates aerobic degradation of pollutants. The practice is generally limited to superficial soil treatment (10–35 cm). Much attention has been given to this technology because cleanup liabilities, costs of maintenance, and monitoring are reduced (Vidali 2001). Moisture content, aeration, pH and amendments such as nutrient, and bulking agents are the conditions controlled during landfarming (Jain et al. 2011).

10.1.28 Biopiles

Biopiles involve hybridizing composting and landfarming. In this technology, engineered cells are fabricated as piles of aerated compost and are used for treating surface polluted with petroleum products. They are landfarming improvised versions, which control pollutants physical losses by volatilization and leaching (Jain et al. 2011). Favorable environment is provided for indigenous aerobic and anaerobic microbes in biopile technology (Vidali 2001).

10.1.29 Bioreactors

Bioreactors are used for offsite treatment of polluted soil and water pumped up from a contaminated groundwater plume. Processing of polluted sediment, soil, water, or sludge involves the use of engineered vessel (bioreactor). The extent or rate of pollutant degradation in bioreactors is mostly greater than in in situ pollutant degradation techniques because the reactor is more easily controlled and predicted. Despite the advantages of bioreactors, the polluted soil needs to be pretreated before it is treated in a bioreactor (Kumar et al. 2011).

10.2 Sample Collection and Preliminary Analysis of the Soil Sample

The naturally polluted surface soil (0–10 cm) with UMO was collected in a black polythene plastic bag from Old Dan Gombe Auto-Mechanics Workshop located along Jos Road, Bauchi State, Nigeria. The collected sample was stored at 4 °C in a refrigerator while awaiting microbial analysis. The horse manure was sourced from horse stable in Kobi Street Bauchi, Bauchi State, Nigeria; elephant dung from Yankari Game Reserve Bauchi State, Nigeria; and donkey dung from Gwallameji Village opposite Federal Polytechnic Bauchi, Nigeria.

Various physicochemical and microbial analyses were conducted on the polluted soil sample. The pH was determined using the method reported by Abdulsalam (2012), the organic carbon was determined according to Walkley and Black (1934), and moisture content was determined as described by Motsara and Roy (2008). The particle density and bulk density were determined based on the procedures reported by Lambe and Whitman (1969) and McKenzie et al. (2004) respectively, and their values were used for calculating soil porosity (Abdulsalam et al. 2012). Total nitrogen in the polluted sample was determined by the Kjeldahl method, while available phosphorus was obtained by the spectrophotometric method (Abdulsalam et al. 2012). Characterization and identification of the pure bacterial isolate were conducted using the standard procedure in Bergey's manual (Ishak et al. 2011). The oil and grease content was determined using Soxhlet extraction method (Ebenezer 2013), and total heterotrophic bacterial count was obtained by inoculating 0.1 ml of the serially diluted sample on the nutrient agar (oxoid) plate using the spread plate method (Odokuma and Okpokwasili 1993). All physicochemical and microbiological analyses of the soil samples and different animal manures were conducted in duplicate.

10.2.1 Determination of Oil and Grease Contents and Total Heterotrophic Bacterial Count

The oil and grease content of sample was determined using Soxhlet extraction method (Ebenezer 2013). Purified sodium sulfate (Na_2SO_4) was obtained by drying overnight in an oven at 105 °C. The Soxhlet flask was dried at 105 °C for 30 min before being cooled. The weight of the Soxhlet flask and boiling chip was measured and recorded (W_2). Then, 3.0 g of anhydrous Na_2SO_4 was added to 3.0 g of polluted soil in a cellulose extraction thimble. 60 ml of n-hexane was added to the flask, and the extraction process was allowed to run for 60 min. The residual O&G was determined by evaporating the n-hexane in a hot water bath; the cooled flask was weighed again (W_1). Residual O&G content in the soil was calculated according to Eqs. (10.3) and (10.4).

$$\text{Oil and grease fraction (ppm)} = \frac{\text{The gain in weight of the flask}}{\text{Weight of soil}} \times 1000 \tag{10.3}$$

$$\text{Oil and grease fraction (ppm)} = \frac{w_1 - w_2}{W} \times 1000 \tag{10.4}$$

W_1 = weight of flask, boiling chips and residue after evaporation of hexane (mg)
W_2 = weight of round flask and boiling chips (mg)
W = weight of the contaminated soil (g)

The percentage degradation (D) of the oil was determined using Eq. (10.5).

$$D = \frac{R_{\text{O\&Gi}} - R_{\text{O\&Gr}}}{R_{\text{O\&Gi}}} \times 100 \tag{10.5}$$

where $R_{\text{O \& Gi}}$ and $R_{\text{O \& Gr}}$ are the initial and residual O&G concentration, respectively.

The total heterotrophic bacterial count present in the treatment cells was determined by spread plate techniques. It was achieved by subjecting the samples to serial dilution, which was plated on nutrient agar (NA) oxoid and incubated at 28 ± 2 °C for 24 h. Plates that yielded count between 30 and 300 colonies were counted (Agarry et al. 2010).

10.2.2 Experimental Design and Treatment

One and a half kilograms (1.5 kg) of UMO-contaminated soil with particle size of less than or equal to 2 mm was mixed with each of horse dung, elephant dung, donkey dung, and the combination of the three in plastic containers (treatment cells) in the ratio of 10% w/w (Agarry et al. 2010; Sarang et al. 2013; Stanley 2013; Victor et al. 2015). Each of the treatment cells was labelled as T2, T3, T4, and T5, respectively. Also, control treatment cells containing polluted soil without stimulant (amendment) were set up. Distilled water was added as needed to adjust the moisture content and maintain it at 20% water holding capacity (Artin 2010). The cells were kept at room temperature (28 ± 2 °C) and aerated by pulverizing the content of each treatment cell twice a week. Samples from each of the treatment cells were taken for weekly analysis, including residual O&G content and microbial count. The experimental procedure was run for 6 weeks period. The setup of the experimental design is shown in Table 10.1.

10.2.3 Response Surface Methodology Optimization of Batch Experimental Data

A total of 28 experiments were generated using a response surface methodology (RSM) design with time as the numeric factor and biostimulant type as the categorical factor. The bioremediation time was in the range of 0–42 days. The bioremediation time (numeric factor) was varied between 0 and 42 days, while the biostimulant type (categoric factor) had four levels: elephant dung (E), horse dung (H), donkey dung (D), and elephant-horse-donkey dung (EHD) combined at the ratio of 1:1:1. The responses of the biostimulation system were % O&G removal and bioremediation efficiency. In the modelling aspect, the obtained experimental data (responses) were entered into the design matrix and analyzed using cubic model since a previous study showed that a cubic model better represented a biochemical system of this type (Giwa et al. 2017). In the optimization step, numerical type was used, in which the goals were in range for the factor and maximize for the responses. The design of experiments, modelling, and numerical optimization were all done using Design-Expert version 13.0.

Table 10.1 Summary of the experimental design

Treatment cells	Description
T_1	1500 g contaminated soil
T_2	1500 g contaminated soil +10% horse dung
T_3	1500 g contaminated soil +10% elephant dung
T_4	1500 g contaminated soil +10% donkey dung
T_5	1500 g contaminated soil +10% mixture of the three dung

10.3 Physicochemical Properties of Soil and Organic Wastes

For this study, the UMO-contaminated soil and animal dung properties (physical and chemical) are given in Table 10.2. In the polluted soil, total organic carbon was high with a value of 24.74% because the oil and grease (O&G) content of the UMO in the soil was found to be 112,734 mg kg^{-1}. This value exceeds the Nigeria Ministry of Environment safe limit (500 mg kg^{-1}) (Abdulsalam et al. 2011). Thus, it is a necessity for the polluted soil to be remediated. Vidali (2001) observed that for effective bioremediation, the acceptable pH limit is 5.5–8.5, and the soil pH was found to be within this limit with a value of 6.9. The soil moisture content (2.57%) fell out of the limit of 12–25% required for optimum growth, and proliferation of microbes (Adams et al. 2015) observed that for optimum microbial proliferation and growth, the optimum moisture content must be within 12–25%, and the moisture content was found to be out of this limit (12–25%) with a value of 2.57%; thus, the soil moisture content must be adjusted. The polluted soil was amended with animal wastes (horse, donkey, and elephant dung) because of the soil's low nitrogen content of 0.42%. For a stimulant to remediate polluted soil effectively and efficiently, one of the limiting nutrients (nitrogen of phosphorus) must be present. Nitrogen contents of 0.67%, 0.98%, and 1.96% were found to be present in donkey, horse, and elephant, respectively.

10.3.1 Total Heterotrophic Bacterial Count in the Contaminated Soil

The UMO-contaminated soil total heterotrophic bacteria count (THBC) is given in Table 10.3 and was found to be 9.10E+08 CFU g^{-1} soil. The polluted soil has high concentration of indigenous microbe whose value (9.10E+08 CFU g^{-1} soil) exceeded

Table 10.2 Physicochemical properties of the sample

	Samples			
Parameter	Soil	Horse dung	Elephant dung	Donkey dung
pH	6.9	6.8	6.9	5.8
Nitrogen (%)	0.42	0.98	1.96	0.67
Organic C (%)	24.74	10.51	4.79	6.92
Phosphorus (%)	0.67	0.46	0.21	0.16
Moisture (%)	2.57	ND	ND	ND

Table 10.3 Heterotrophic bacteria count in the contaminated soil

Sample	Total heterotrophic bacteria count (CFU g^{-1} soil)
Contaminated soil	9.10×10^8

the minimum value (1.00E+05 CFU g^{-1} soil) required for bioremediation to take place effectively and efficiently.

10.3.2 Oil and Grease (O&G) for the Treatment Cells

Abdulsalam et al. (2012) used O&G content as a better index for remediation to study the extent of pollutant degradation in UMO-contaminated soil. The total petroleum hydrocarbon was not used because the C–H bond in UMO dropped. The residual oil and grease with bioremediation time in the treatment cells is shown in Fig. 10.3. As the bioremediation time proceeds, the percentage degradation of O&G increased, and this is the observed trend for a typical UMO biodegradation process. For the first 14 days of the bioremediation, the polluted soil O&G content reduced more for all the treatment cells amended (T_5, T_4, T_3, and T_2) than the unamended one (T_1) (Fig. 10.4). The concentration of the O&G content reduced from 104,285, 104,469, 104,642, 103,149, and 112,738 mg kg^{-1} to 59,536, 52,596 41, 756, 42,427, and 89,450 mg kg^{-1} in treatment cells T_5, T_4, T_3, T_2, and T_1, respectively, after 14 days of the study. This reduction in O&G content amounts to 52.2, 60.8, 71.4, 66.9, and 29.5% reduction. After 42 days of the study, the O&G content concentration reduced to 34,131, 31,997, 17,025, 21,265, and 63,655 mg kg^{-1} in treatment cells T_5, T_4, T_3, T_2, and T_1, respectively, with a corresponding percentage O&G reduction of 67.3, 69.4, 83.74, 79.40, and 43.50% for treatment cells T_5, T_4, T_3, T_2, and T_1, respectively. It was observed that UMO degradation in T_5, T_4, T_3, and T_2 was more efficient with T_3 having the highest O&G removal with a value of 83.7%.

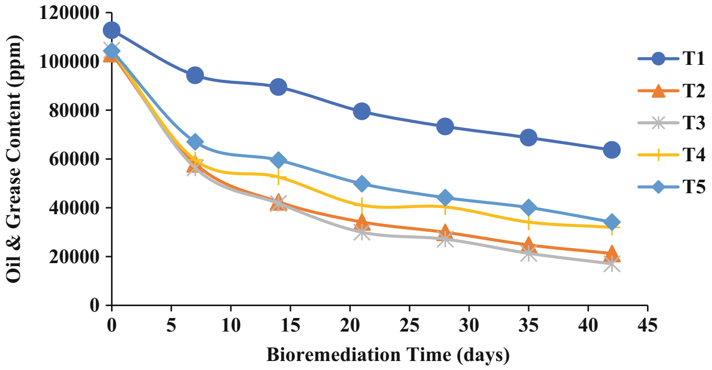

Fig. 10.3 Variation of oil and grease (mg/kg) with bioremediation time (days)

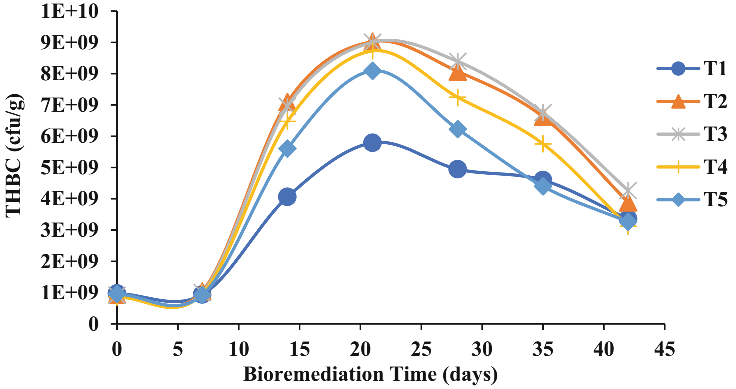

Fig. 10.4 Variation of total heterotrophic bacteria count (THBC) with bioremediation time

10.3.3 Microbial Analysis

Figure 10.4 presents the total heterotrophic bacteria count results for the entire period of the study. As observed from the figure, the growth profile of the microbes followed a typical growth pattern of microorganisms with death, stationary, log/exponential, and lag phases. Similar lag phase trend was shown by the polluted soil in treatment cells with and without amendment. Microbes adapt to the new environment in his phase for 7 days. Similar log/exponential phase pattern was followed from 7 to 21 days with maximum UMO degradation. After incubation period of 42 days, the treatment cells T_5, T_4, T_3, T_2, and T_1 THBC were 3.57E+09, 4.26E+09, 3.88E+09, 3.35E+09, and 2.36E+09, respectively. This microbial growth trend observed is similar with O&G percentage degradation, and this confirms that microbes utilized the UMO as their source of energy and carbon.

10.3.4 Bioremediation Kinetic Model Fitting

The process of biodegradation of UMO in all the treatment cells was investigated using first-order and second-order kinetics (El-Gendy and Farah 2011; Abioye et al. 2012; Agarry and Latinwo 2015; Agarry and Oghenejoboh 2015; Abdulyekeen et al. 2016). The stimulants' (animal wastes) effectiveness in enhancing the UMO degradation in all the treatment is compared by evaluating the biodegradation half-life and rate constant. The first- and second-order kinetic model expressions are given by Eqs. (10.6) and (10.7), while the expressions for calculating the biodegradation process half-life ($t_{1/2}$) in days are given by Eqs. (10.8) and (10.9).

Table 10.4 Summary of the samples rate constants, half-lives, and correlation coefficients for first and second order

	First order			Second order		
Sample	k_1 (day^{-1})	$t_{1/2}$ (day)	R^2	k_2 (kg/ mg/day)	$t_{1/2}$ (day)	R^2
T_1	0.013	53.3	0.9763	2E-07	47.1	0.9920
T_2	0.035	20.0	0.9287	9E-07	9.9	0.9958
T_3	0.023	17.4	0.9463	1E-06	9.7	0.9863
T_4	0.026	27.5	0.8718	5E-07	19.7	0.9573
T_5	0.024	29.0	0.9358	4E-07	22.0	0.9856

$$\ln R_{\mathrm{O\&Gr}} = -k_1 t + \ln R_{\mathrm{O\&Go}} \tag{10.6}$$

$$\frac{1}{R_{\mathrm{O\&Gr}}} = k_2 t + \frac{1}{R_{\mathrm{O\&Go}}} \tag{10.7}$$

$$t_{1/2} = \frac{\ln 2}{k_1} = \frac{0.693}{k_1} \tag{10.8}$$

$$t_{1/2} = \frac{1}{k_2 R_{\mathrm{TPHO}}} \tag{10.9}$$

where $R_{\mathrm{O\&Gr}}$ and $R_{\mathrm{O\&G0}}$ are O&G residual and initial concentration (mg kg^{-1}), respectively, t is the bioremediation time (day), while k_1 (day^{-1}) and k_2 (kg/mg/day) are the rate constants of the biodegradation process for first-order and second-order kinetics. The kinetic parameters obtained from the two models (first and second) are given in Table 10.4. To know the model that best fit the experiment data, the linear regression coefficient of determination (R^2) was evaluated. The R^2 values for all the treatment cells range from 0.8718 to 0.9907 and 0.9485 to 0.9958 for first- and second-order kinetics, respectively. The hydrocarbon degradation in the treatment cells is best described by the model with relatively high R^2 value.

Results presented in Table 10.4 revealed that the biodegradation of UMO in soil spiked with elephant dung (T_3) had a lower half-life and higher rate constant for both first order ($k_1 = 0.0399$ day^{-1} and $t_{1/2} = 17.37$ days) and second order ($k_2 =$ 1E-06 kg/mg/day) compared to soil spiked with other animal wastes.

10.3.5 Bioremediation Efficiency

The effectiveness of the biostimulants was compared by evaluating the bioremediation efficiency using Eq. (10.10).

$$\%\mathrm{Efficiency\ (Eff)} = \frac{\%R_{(a)} - \%R_{(u)}}{\%R_{(a)}} \times 100 \tag{10.10}$$

Table 10.5 Percentage degradation of used oil and bioremediation efficiency of the stimulants after 42 days of bioremediation

Treatments cells	O&G degradation (%)	Eff (%)
T_1 (Control)	43.54	–
T_2 (Horse dung)	79.38	45.16
T_3 (Elephant dung)	83.73	48.01
T_4 (Donkey dung)	69.37	37.24
T_5 (Their combination)	67.27	35.28

where $\%R_{(a)}$ is the percentage degradation of oil and grease content in the amended soil and $\%R_{(u)}$ is the percentage degradation of oil and grease content in the unamended soil. The results of the Eff (%) are presented in Table 10.5. Based on the result, it was found that soil amended with elephant dung (T_3) is more effective than soil amended with horse dung (T_2), donkey dung (T_4), and their combination (T_5).

10.3.6 Batch Data Statistical Modelling and Optimization

The single variable with four-level categorical factor design matrix obtained for the aim of studying the synergistic effect of time and biostimulant type on O&G remediation and its efficiency is given in Table 10.6.

There are 28 experimental runs in the design matrix with seven runs for each labelled as elephant (E), horse (H), donkey (D), and elephant-horse-donkey (EHD) stimulants. As it can be noticed from Table 10.6, variation of time and type of biostimulant used had effect on the responses. Increasing the bioremediation time led to increase in O&G removal for all the biostimulants. However, the maximum O&G removal was achieved with E dung, although this maximum value was not efficient enough when compared to attenuation. Also, this trend is evident in Fig. 10.5. The improved O&G removal can be associated with the fact that one of the nutrients needed for growth of organism is nitrogen; this means that when it is present in high concentration, the growth rate is expected to be favored (Abioye et al. 2009, 2012; Agarry and Jimoda 2013; Agbor et al. 2018). Besides, the E dung had the lowest quantity of total organic carbon; this proved that the microbial rate of poisoning was very low. The analyses of variance results are obtained when the cubic model was used for the analyses of the experimental data presented in Tables 10.7 and 10.8.

The cubic model was discovered to better represent the experimental data as evident in the probability of error value (p-value) < 0.0001 on 95% confidence level. For O&G removal, both factors (bioremediation time and biostimulants type) singly affected the response as evident in the p-values of this model terms being less than 0.0001 (Table 10.7). Also, the R-squared value of the model was close to unity (0.9875). The predicted R-squared value of 0.9431 is in reasonable agreement with the adjusted R-squared value of 0.9774; that is, the difference is less than 0.2. The high R-squared value can be clearly observed in the way in which the predicted

Table 10.6 Design matrix for biostimulation of UMO contaminated soil

		Factor 1	Factor 2	Response 1	Response 2
Std	Run	A/A	B/B	O&G removal	BE
				%	%
1	25	0	ES	0	0
2	8	7	ES	46.17	64.52
3	26	14	ES	60.1	65.63
4	5	28	ES	74.06	52.77
5	2	35	ES	79.62	50.99
6	1	42	ES	83.73	48
7	20	21	ES	71.36	58.65
8	14	0	HS	0	0
9	3	7	HS	43.74	62.54
10	18	14	HS	58.87	64.91
11	10	28	HS	70.98	50.71
12	19	35	HS	76	48.66
13	28	42	HS	79.38	45.16
14	12	21	HS	66.87	55.88
15	17	0	DS	0	0
16	15	7	DS	42.93	61.84
17	16	14	DS	49.65	58.4
18	9	28	DS	61.37	43
19	23	35	DS	67.35	42.06
20	24	42	DS	69.37	37.24
21	13	21	DS	60.75	51.43
22	11	0	EHDS	0	0
23	27	7	EHDS	35.75	54.18
24	21	14	EHDS	42.91	51.87
25	6	28	EHDS	57.67	39.33
26	22	35	EHDS	61.55	36.6
27	7	42	EHDS	67.27	35.28
28	4	21	EHDS	52.2	43.47

O&G removal correlates with the experimental values as shown in Fig. 10.6. In addition, normal plot of residuals indicates that the errors (residuals) follow the normal distribution as they are almost fairly distributed along the straight line (Fig. 10.7). The cubic model in terms of actual factor for O&G removal is given by Eqs. (10.11a), (10.11b), (10.11c), and (10.11d). This equation is useful for making predictions about the response for the given levels of each factor and for identifying the relative impacts of the factors by comparing the factor coefficients.

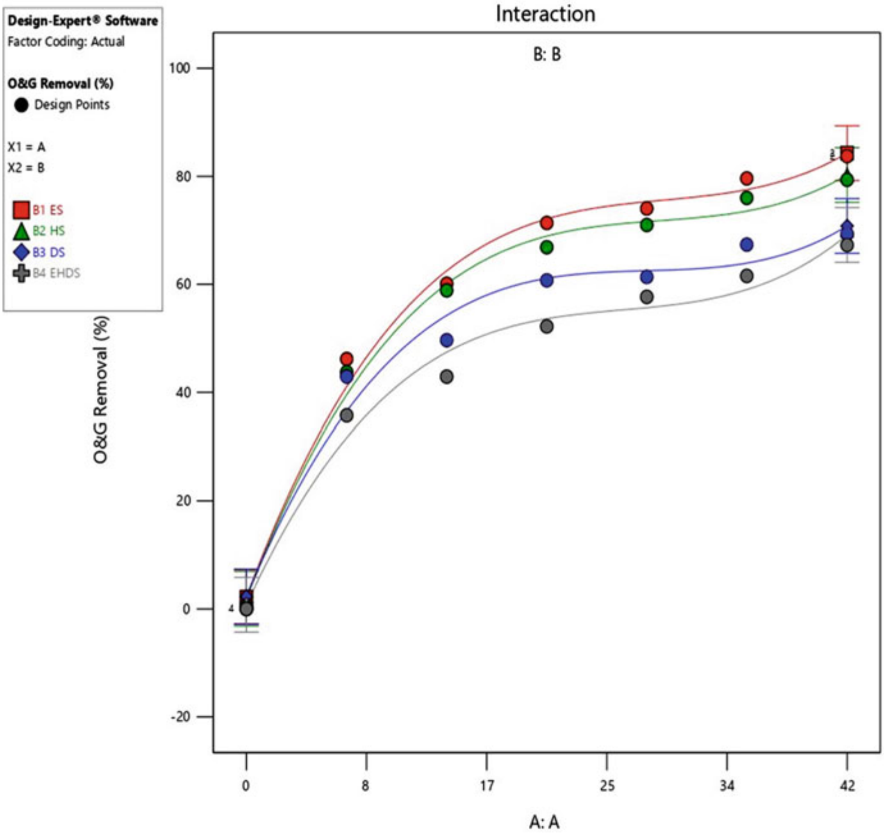

Fig. 10.5 The effect of time and biostimulant type on O&G removal

Table 10.7 ANOVA for O&G removal cubic model

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	16828.22	12	1402.35	98.44	< 0.0001	Significant
A-A	12512.31	1	12512.31	878.27	< 0.0001	
B-B	830.50	3	276.83	19.43	< 0.0001	
AB	113.35	3	37.78	2.65	0.0865	
A^2	2590.82	1	2590.82	181.86	< 0.0001	
A^2B	65.77	3	21.92	1.54	0.2455	
A^3	715.47	1	715.47	50.22	< 0.0001	
Residual	213.70	15	14.25			
Cor total	17041.92	27				

Table 10.8 ANOVA for BE cubic model

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	9880.06	12	823.34	11.80	< 0.0001	Significant
A-A	873.58	1	873.58	12.52	0.0030	
B-B	552.31	3	184.10	2.64	0.0875	
AB	63.10	3	21.03	0.3015	0.8238	
A^2	4950.56	1	4950.56	70.97	< 0.0001	
A^2B	46.18	3	15.39	0.2207	0.8805	
A^3	3394.32	1	3394.32	48.66	< 0.0001	
Residual	1046.37	15	69.76			
Cor total	10926.44	27				

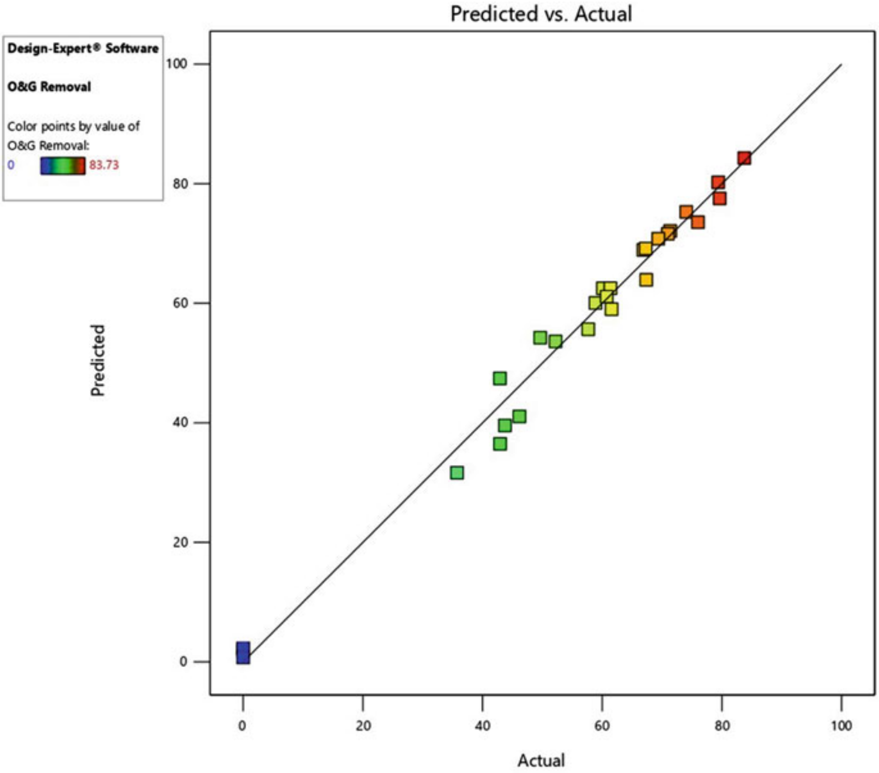

Fig. 10.6 Predicted and actual (experimental) O&G removal

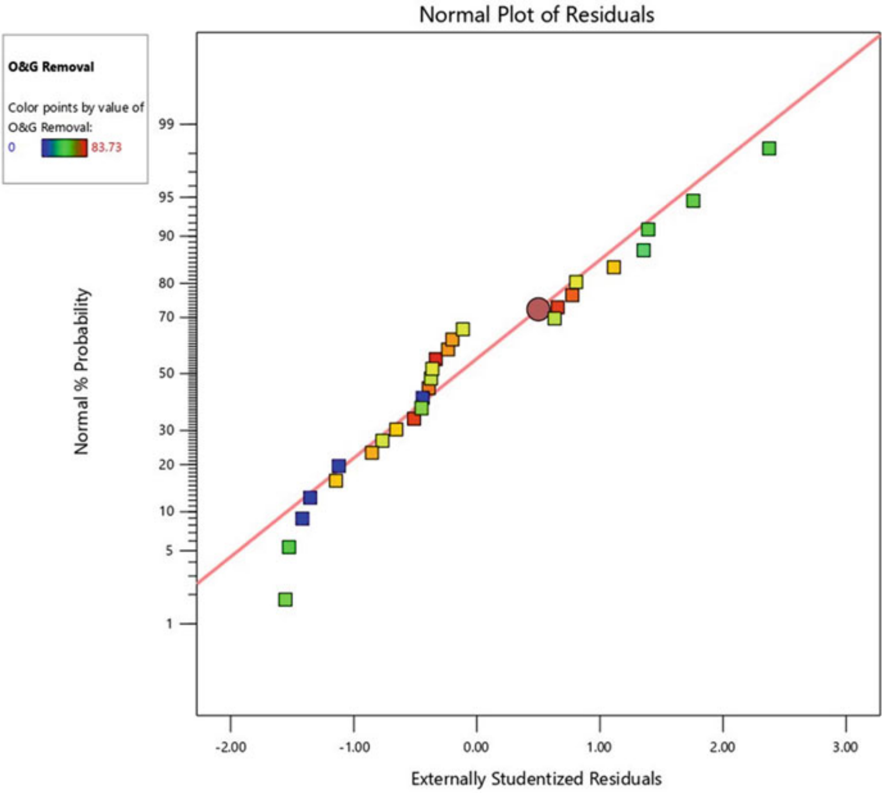

Fig. 10.7 Normal plot of residuals for O&G removal

$$\mathrm{O\&G(E)} = +2.20231 + 7.04181 * A - 0.232510 * A^2 + 0.002652 * A^3 \tag{10.11a}$$

$$\mathrm{O\&G(H)} = +1.85608 + 6.86035 * A - 0.230289 * A^2 + 0.002652 * A^3 \tag{10.11b}$$

$$\mathrm{O\&G(D)} = +2.29165 + 6.30977 * A - 0.222783 * A^2 + 0.002652 * A^3 \tag{10.11c}$$

$$\mathrm{O\&G(EHD)} = +0.756297 + 5.74565 * A - 0.209413 * A^2 + 0.002652 * A^3 \tag{10.11d}$$

Furthermore, the ANOVA result given in (Table 10.8) shows that the BE model obtained was significant with A, A^2, and A^3 as the influencing terms because p-values less than 0.05 on confidence level of 95% were recorded. Also, the R-squared value

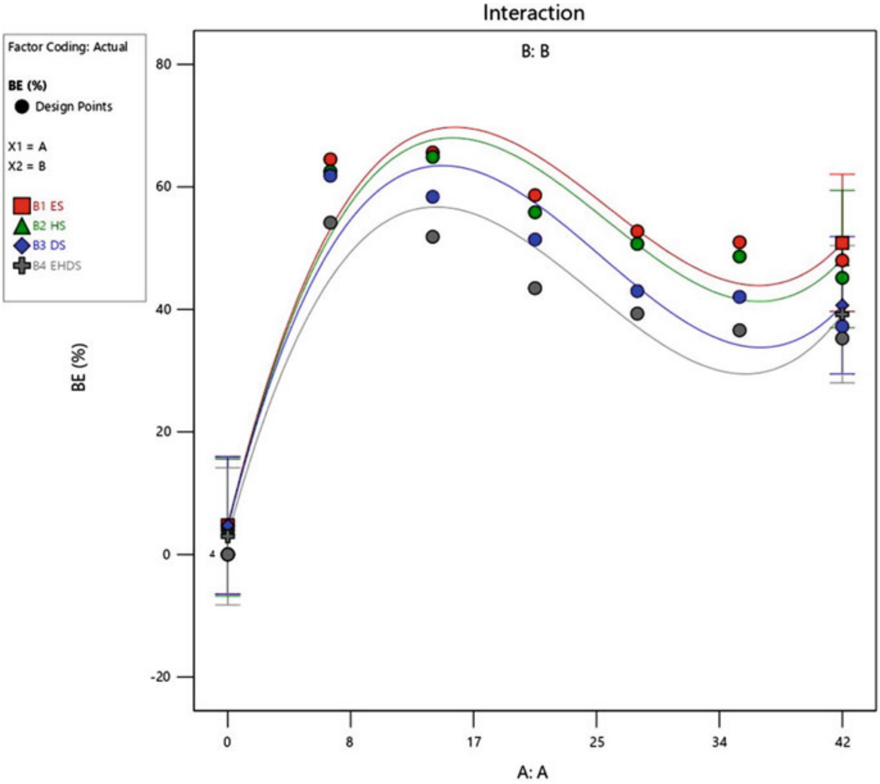

Fig. 10.8 The effect of time and biostimulant type on BE

of the model was close to unity (0.9042). However, the biostimulant type seems not to influence the response (BE) significantly; this was because bioremediation efficiency (BE) depends on bioremediation time naturally, irrespective of the biostimulant type. The attenuation rate tends to increase with increase in bioremediation time. All the same, from Fig. 10.8, the E dung still had the highest BE, although for all the stimulants' categorical level, BE decreased with an increase in bioremediation time. Similar trend of result was observed in the work reported by Giwa et al. (2017) and Ofoegbu (2015). The cubic model in terms of actual factor for BE is given in Eqs. (10.12a), (10.12b), (10.12c), and (10.12d). In summary, the maximum O&G removal and BE was achieved with E dung as biostimulants. These results corroborate the ones reported by Abdulyekeen et al. (2016) and Giwa et al. (2017).

$$\mathrm{BE}\ (\mathrm{E}) = +4.76161 + 9.76303 * A - 0.448940 * A^2 + 0.005777A^3 \tag{10.12a}$$

$$\mathrm{BE}\ (\mathrm{H}) = +4..39037 + 9.65583 * A - 0.447671 * A^2 + 0.005777A^3 \tag{10.12b}$$

$$\mathrm{BE\ (D)} = +4.73036 + 9.25552 * A - 0.442607 * A^2 + 0.005777A^3 \quad (10.12c)$$

$$\mathrm{BE\ (EHD)} = +2.97064 + 8.72523 * A - 0.429814 * A^2 + 0.005777A^3 \quad (10.12d)$$

10.3.7 Optimization Findings

Traditionally, optimization is considered a very important tool that helps in making decision concerning processes. The RSM optimization carried out showed that only with elephant dung as biostimulant, the bioremediation led to 85.4% O&G removal with BE of 51.7% at 42 days. This result was almost similar to the value obtained experimentally at week 6 (42 days) of the study (Table 10.8).

10.4 Conclusion

For this study, the potential of biostimulation using horse, elephant, donkey, and the mixture of the three dungs were investigated in five treatment cells to remediate UMO-contaminated soil. The reduction of residual UMO and the microbial growth data after 6 weeks (42 days) of the study showed that the degradation of UMO occurred and indigenous heterotrophic bacteria population increased in all the treatment cells. Results obtained showed that the application of horse (T_2), elephant (T_3), donkey (T_4), and the mixture of the three dungs (T_5) stimulated the indigenous microorganisms in the polluted soil with O&G percentage degradation of 79.38%, 83.73%, 69.37%, and 67.27%, respectively. Elephant dung is the most effective stimulant compared to horse, donkey, and the mixture of the three dungs. The evaluated rate constants and half-lives of the studied kinetics (first and second order) showed that the soil spiked with elephant dung had the least half-life values of 9.7 days and 17.4 days for first and second order, respectively, as well as the highest rate constant value of 0.0399 day^{-1} and 1E-06 kg/mg/day for first and second order, respectively. Therefore, elephant dung was the most effective biostimulant to horse, donkey, and the mixture of the three. The analysis of variation (ANOVA) results showed that the bioremediation efficiency and O&G removal for the bioremediation process was best represented by cubic model. Furthermore, while O&G removal was affected by biostimulant types and bioremediation time, the BE is only affected by bioremediation time. It was also observed from the numerical optimization carried out that 84.31% O&G removal and 50.86% BE could be achieved with elephant dung as stimulant after 6 weeks (42 days) of bioremediation study.

Acknowledgments The authors thank the management of Abubakar Tafawa Balewa University, Bauchi, for their financial support for this research.

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Chapter 11
Enzymes Involved with Digestion of Animal Nutrition: Role and Their Biotechnological Application

Charles Oluwaseun Adetunji, Olugbemi T. Olaniyan, and Ajit Varma

Abstract Proper knowledge of the digestive exogenous enzymes in animals has been established as a better biotechnological tool that could facilitate their efficiency, growth and developmental pattern, increased metabolism, and performance. This novel technology has been established to accelerate the level of reduction in food insecurity that is believed to affect many parts of the developing countries that cannot afford basic nutrition. Several authors have reported that in the production of ruminant feeds, feeds are exposed to exogenous enzymes like amylase, maltase, β-glucanase, lipase, pectinase, cellulase, protease, tannase, and xylanase which are known to increase the nutritional utilization in livestock through the elevation of fermentation process and reduction in the digestibility lag time. This review intends to provide a detailed information on enzymes involved with digestion of animal nutrition as well as their biotechnological application.

Keywords Methanogens · Endoparasites · Methanogenic archaea · Biogenic methane · Real-time PCR

11.1 Introduction

The request for livestock products has increased drastically around the globe, and this could be attributed to the higher level in the human population as a consequence of rise in urbanization and income growth (Thornton 2010). It has been established that majority of the food ingested in most developing countries is majorly

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_11

contributed by mixed crop-livestock producer or small-scale as well as pastoral farmers John and Maria (2001). Interestingly, biotechnology has been reorganized as a sustainable novel technology that could lead to increase in food security (Adetunji and Anani 2021a, b; Okeke et al. 2021; Adetunji et al. 2021a, b, c, d; Adetunji et al. 2020; Olaniyan and Adetunji 2021) and its safety that the utilization naturally available resources Bimrew (2014).

The application of biotechnology for the production of animal feed could enhance the quality through the release of more amino acids and fat content. The application of enzymes could enhance the nutritional contents present in the animal feed and decrease the loss of feed, destruction of pathogenic microorganisms, generation of better fodder and feed that possess better nutritional values, and increase in the activities of probiotics and prebiotics (Shelke et al. 2011; Bimrew 2013).

Moreover, the digestibility of the animal could also be improved through the addition of enzymes in their feed and enhanced milk and egg production as well as production of better genetic selection for feed efficiency, enhanced development, carcass quality, and reproduction (Capper 2011; Meyer et al. 2006; Klusmeyer et al. 2009; Connor 2015). Therefore, this chapter intends to provide a detailed information on the application of enzymes involved with digestion of animal nutrition as well as establish their biotechnological application.

11.2 Different Enzymes Involved with Digestion of Animal Nutrition

Menezes-Blackburn and Greiner (2015) reported that in the production cost of monogastric animals, feed is the highest expenses; thus many research have tried to identify different enzymes that can be deployed to significantly address and increase the efficiency and production of feeding pattern. The authors discovered that these enzymes are currently being utilized to suppress antinutritive factors, enhance feed digestive constituents, facilitate low-cost ingredients, lower viscosity in the gut, and manage waste disposal and risk related to manure. Different feed enzymes are identified such as xylanases, β-glucanases, mannanases, pectinases, α-galactosidases, amylases, phytases, and protease. It has been reported that these enzymes are the main market value for the future feed enzymes due to the ever-increasing industry particularly poultry. de Souza Moura et al. (2014) reported that in recent years, several enzymes involved in animal diets have been identified to help in advancing animal nutrition and health due to the development and discovery of several other ingredients linked to them. The authors noted that in the production of eggs, meat, and fish, conventional feedstuffs are being utilized like corn, soybean, and millets which have been reported to become very expensive in the nearest future. Thus, new ingredients will become very attractive and as alternatives to lower the cost of production and increase the health of these animals. Addition of enzymes has been reported to offer many advantages such as improving feeding efficiency, improving digestibility, and lowered pollution load from animals. The authors highlighted the following exogenous enzymes like cellulase, pectinase, xylanase, hemicellulase, phytase, protease, glucanase, amylases, galactosidases, lipases, and phytases in improving physiological functions and performance.

de Souza Moura et al. (2014) emphasis the relevance of some enzymes that are capable of fermenting and degrade complex structure in forage, fiber in the host animals because of the high level of degradation potential of these enzymes. Salem et al. (2015) reported that different cocktail of developed enzymes was utilized for the digestion, fermentation, and degradation of complex forage constituents in the Barki sheep fed with *Atriplex halimus*. The authors noted that f ZAD1 (Z1) and ZAD2 (Z2) are the liquid enzyme preparations and ZAD0 (Z0) is the powder preparation. From their experiments, it was discovered that these enzymes were able to increase digestibility, improve feeding intake and nitrogen utilization, and balance together with enhancing fermentation process.

Imran et al. (2016) reported that enzyme supplementation in animal feeding and nutrition have been seen to display specific roles to improve the overall nutritional value, efficiency, and eliminating antinutritive contents present in the ingredients. Hence, this will reduce the overall cost of production and maintain gut health and growth pattern. The authors highlighted the roles of phytases, beta-glucanase, proteases, cellulases, and alpha-amylases in pharmaceutical, food, drink, and animal feed industry. So many authors have reported the need for enzyme technology in animal feed such as poultry and pig diets. The cost of production in this sector seems to be high, and thus these enzymes will enhance digestibility, improve microflora growth, reduce the nutrients availability to gut microflora, suppress pathogenic organisms, lower gut viscosity increasing nutrient absorption, eliminate bacteria growth, and reduce ammonia buildup.

Devant et al. (2020) reported that the utilization of exogenous glucoamylase enzymes and in combination with a neutral protease on feces D-lactate and apparent total tract digestibility in crossbred angus bulls fed a ration rich in rolled corn. The authors reported that enzyme supplements utilized as feed additive are capable of increasing feed efficiency and increase economic benefits.

Zargi (2018) demonstrated that β-glucanase and xylanase as feed additives to improve the nutritive value of poultry feed cereal diet increased the performance of poultry. These exogenous enzymes are linked to reduce intestinal viscosity through prebiotic effects, thus generally increasing the productivity, increasing the availability of nutrients, reducing antinutritive constituents, and increasing nutritive value and performance of the animals.

Bimrew (2014) reported that several nutritive enzymes are currently utilized for enhancing greater performance in monogastric animals. These exogenous digestive enzymes increase the animal's digestibility and increase feeding efficiency and general ability to breakdown dietary fiber, starch, phytate, and protein. The general physiological principles on the role of these exogenous enzymes on the gut microbiota are to encourage the elevation of digesta viscosity to achieve slow diffusion rate and increased accumulation of nutrients. Feeding has been seen to constitute the larger part of livestock production cost; thus feeding efficiency will remain the main focus of many farmers to be economically sustainable. Though the utilization of many exogenous enzymes in ruminant diets have not been greatly harnessed, several authors have highlighted the beneficial advantages in the promotion of animal's performance and nutritive value. The exogenous enzymes are generally classified into three different types like the proteolytic enzymes, fibrolytic enzymes, and the amylolytic enzymes. There are many factors that influence the activities of these exogenous enzymes such as species, enzyme utilized, physiological status, gut conditions, rumen pH, nutrient composition of diets, and enzyme dosage (Roque et al. 2017).

de Lima et al. (2012) reported the utilization of supplemented enzyme complex and formulation diet to reduce protein content and increase productivity in laying hens. From their reports, it was discovered that the supplemented enzyme complex significantly improves the performance lever in the layer and increase the egg quality, gut health, and mineralization in the bones. According to the authors, it was analyzed that addition of enzymes to the feeding pattern of animal will ultimately increase the digestibility, enhance intestinal physiology, and lower digesta viscosity.

Khattak et al. (2006) revealed that exogenous enzymes are capable of breaking down complex fiber into nutritive constituents, thus increasing the biochemical, metabolic, and physiological mechanisms involved in feeding process. Yang et al. (1999) highlighted the role of exogenous enzymes as feed additive in improving the milk production and digestibility capacity in lactating dairy cow. In their study, it was identified that these fibrolytic enzyme supplementation like xylanase and cellulase increased the cow's milk production, composition, and digestion process in the dairy.

Bedford (2018) revealed that several enzymes are applied and utilized for improving feeding industry and other multi-enzyme products which involves mechanisms like prebiotic activity, reduced viscosity, destruction of cell wall, and enhanced digestibility.

Walsh et al. (1993) reported that due to the high economic value placed on livestock animals, there is increased demand and pressure resulting in various utilizations of chemical substances and growth-promoting drugs in animal production. This has led to various laws and legislation banning the use of these toxic compounds. Hence, the utilization of animal diet enzyme supplements containing hydrolytic enzymes has become highly attractive to many animal producers for increased nutritional efficiency, performance, growth, and development. Generally, animals use the binding of specific substrate in animal feed by enzymes so as to breakdown compounds into smaller molecules. This specific substrate enzyme interaction is highly efficient at a very optimal temperature and pH. Reports suggest that many enzymes are added to animal feed referred to as exogenous enzymes which are produced through microbial fermentation process derived from animals, plants, and microorganisms utilized as feed additives. These exogenous enzymes are known to have very low toxicity particularly to the environment and the animals, thus making them very useful as chemically derived animal feed additives. Many of them are capable of improving animal yield, reducing the use of antibiotics, increasing nutritive value, and overall reducing the waste generation. Through the use of biotechnology, scientists have engaged in the production of very high-quality, efficient, and quantity exogenous enzymes at industrial scale to increase animal performance such as carbohydrases, phytases, and proteases.

Bimrew (2014) reported that in animal digestive process, endogenous enzymes alone may not be effective enough to produce the desired result of digestion, thus resulting in reduced performance and yield. Therefore, it is very necessary to constantly supplement animal feed with exogenous enzymes feed additives so as to increase the digestibility and economic benefits associated with the utilization of exogenous enzymes in animal nutrition. This process will improve egg production, meat quality, nutritional profile, increased energy utilization, and digestibility. Studies have revealed that many of these exogenous enzymes are known to improve gut health through the increase in digesta viscosity, reduced diffusion, breakdown of antinutritive compounds, increase in microbial adhesion, reduced shedding of microorganisms, and immune defense. Recently, studies have reported an increase in the global utilization of exogenous enzymes in poultry, ruminant animals, and swine. The continuous use of exogenous enzymes in ruminant animal production will continue to witness sustainability and environmental and economic benefits through improved understanding of feed enzymes in the enhancement of efficiency and performance of ruminant animal production (Yin et al. 2010). The role of digestive enzymes in ruminant animals in the metabolism of constituents, reproduction, and adequate growth will help to generate adequate information about the dietary strategies and nutritional intervention on various enzymatic functions. Recently, it was estimated that by the year 2050, the global cattle production will increase to about 2.6 billion and sheep and goat will increase to 2.7 billion, thus creating a huge burden on the environment through the production of waste with greenhouse gases emission and the use of large land mass for the production of ruminant feed. Therefore, exogenous enzymes may play a critical role in solving many of these problems (Belyea et al. 2010).

Velázquez-De Lucio et al. (2021) reported that exogenous enzymes as animal feed additives have gained a lot of interest among food industries, scientists, and farmers utilizing different approaches particularly bioinformatics tools to design substrate that will facilitate improvement in animal performance.

11.3 Mechanism of Action of Exogenous Enzymes

The mechanisms of action of many of these exogenous enzymes include degradation of anti-nutritional factors, cell wall rupture, increase in the viscosity of feed, hydrolysis by endogenous enzymes, release of nutrients attached to the cell wall, reduction in secretions, digestion of nutrients, loss of endogenous intestinal proteins, digestibility of forage cell walls, mechanical alterations and physiochemical adjustment like gelatinization and hydration, and increase in digestive enzymes. These enzymes can serve as multifunctional catalysts in the improvement and enhancement of nutritional quality of feed through computational methods like next-generation sequencing technologies, bioinformatics, genomic, proteomic, nuclear magnetic resonance spectroscopy, transcriptomic, X-ray crystallography, metabolomics, and several databases or models.

Many exogenous enzymatic nutritional regulations are seen to control metabolic processes in ruminant animals such as enhancing mucosa enzymes, glucose concentration, lipid metabolism, and energy intake. Also due to the fact that many monogastric animals lack some digestive enzymes, adequate compensation must be met through the exogenous enzymes feed additives which can enhance the economic and nutritional aspect of animal production through improved nutrition.

Recent studies have indicated that advancement in molecular biology techniques and biotechnology like genetic tools will help to introduce the much desired enzymes into feeds for regulatory purposes due to the fact that one enzyme may not be effective compared to multiple enzyme action. Simon (1998) reported that non-starch polysaccharide (NSP) hydrolyzing enzymes are being utilized as feed additives which basically depend on so many factors. The action of these enzymes includes decrease of digesta viscosity, nutrient absorption, digestion, metabolic reactions, and digestive tract morphological-histological alterations. Pacheco et al. (2016) reported that many exogenous enzymes were analyzed and tested for their role in feather production, energy level, feather meal, and utilization in adult dogs. In their study using dogs supplemented with diets containing two levels of feather meal, with or without enzyme blend comprising protease and lipase activity, it was discovered that inclusion of enzymes during the feather hydrolysis process significantly enhanced the energy content of the feather meal in diets for adult dogs.

11.4 Conclusion and Future Recommendation

This chapter has established the biotechnological potential and effectiveness of different enzymes involve information on enzymes involved with digestion of animal nutrition as well as their biotechnological application. It has been validated during this study that enzymes could be led to increase in food production and improve digestibility of animal feed. The application of gene-based technologies could also provide more insight on how animal production and provision of better nutrition could be improved. It has also been showed that fibrous feeds such as crop residues, of low digestibility, could be turned into a better animal product.

There is a need for government, policy makers, and relevant stakeholders to support the application of biotechnology at various institutions and industries for effective production of better animal feeds. This will go a long way in supporting increase in food security and better production of animal product as and their feeds. It has also been validated that the application of biotechnology will go a long way in the transformation of naturally available bioresources and their application in resolving several challenges associated with production of animal feed.

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Chapter 12
Life Cycle Assessment: A Novel Approach for Assessment of Carbon Emission at the Farm Gate

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Abstract Greenhouse gas emissions (GHG) from livestock production have a major impact on the environment. Livestock is responsible for approximately 18% of total global GHG emissions. The chapter focuses on applying the life cycle assessment (LCA) approach to examine the emission load of foods from various farming production systems. The LCA of dairy farms is a tool for determining the environmental impact of GHG emissions and emission intensity (Ei) for milk production with a system boundary from "cradle-to-farmgate" or "cradle-to-retailer." The emissions were calculated using whole-farm GHG models based on IPCC methodology with a yearly time-step or country-specific default emission parameters. This method can be summarized as a collection of all inputs and outputs during the production process with the environment. These inputs and outputs would then reveal the impact on the environment. The LCA comprises four sequential phases: definition of objectives and scope, inventory analysis, impact assessment, and result interpretation. The LCA for agricultural GHG emissions is generally based on the requirements and guidelines specified in ISO 14040 to 14044. The article aims to assess carbon footprint from cradle to farm gate following LCA for per kg milk production.

Keywords Global warming · Greenhouse gases · Life cycle assessment · Carbon footprint methane emission

12.1 Introduction

Presently, the agriculture sector is among the most critical anthropogenic activities having a worldwide impact. With the expanding global human population, there is a greater need for natural ecosystems, their conversion to agricultural land, and the increase of cultivation on prevailing agricultural land. We cannot expect a spontaneous trend reversal of increasing environmental burden in the near future since

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_12

population expansion, meat consumption, and agricultural energy use continue to overgrow (Schau and Fet 2008). The rising environmental burden influences soil, water, biodiversity, and the atmosphere. Climate change and its anthropogenic role have become a hot topic in recent years. According to the International Panel on Climate Change (IPCC), human activities trigger the pace of climate change, notably indicated by rapid rising atmospheric concentrations of long-standing global warming gases (IPCC 2007). At the same time, this activity is one of the sustainability goals; only the anthropogenic portion of changes, mainly GHG emission generation, may be regulated.

Climate change substantially impacts global agricultural systems and can be a critical component in ensuring long-term food production. Agriculture is not the primary cause of rising GHG concentrations in the atmosphere, but it is a major contributor. Following energy production, industry, and forestry, agriculture is expected to be the fourth largest source of GHG globally (IPCC 2007). On the other hand, animal farming is thought to be significantly more destructive to global climate warming, owing to research released by the FAO (2015) that identified livestock responsible for 18% of worldwide GHG emissions. According to FAO (2015), the six largest GHG emitting countries are China (29%), followed by the USA (15%), European Union (11%), India (6%), Russia (5%), and Japan (4%). According to the IPCC (2007), GHG emissions increased from 28.7 Gt CO_2-eq/year in 1970 to 49.0 Gt CO_2-eq/year in 2004. Total GHG emissions from livestock are predicted to be 7.1 Gt CO_2 equivalent per year (FAO 2015). At the global level, GHG emissions by the livestock sector consist of enteric fermentation (39%), manure management (26%), feed (21%), land-use change (9%), energy, and post-farm activities (5%), respectively (FAO 2015). In India agricultural sector contributes 19% of GHG emissions (IPCC 2007), in which livestock contributes 54% via enteric fermentation and 2.7% from manure management (INCCA 2010).

Since substantial dramatic episodes are predicted in the future, there is a growing public awareness regarding the impacts of global warming on human health, water supplies, food supply, and several other environmental issues. Methane (CH_4), nitrous oxide (N_2O), and CO_2 are the three primary GHGs emissions recognized by the IPCC to the agricultural sector. GHG is produced in CH_4 from enteric fermentation (EF), N_2O from nitrogenous fertilizers, and CH_4 and N_2O released from direct manure deposition on pastures in more extensive systems or manure management in intensive farming conditions by livestock operations. Climate change models predict that by 2100, the average global temperature will be 2.6–4.8 °C warmer than in 2010 (IPCC 2014).

Increased amounts of GHG in the atmosphere may contribute to global warming. Climate change is likely to influence animal metabolism and health (Brown-Brandl et al. 2003), milk composition, quality and yield (Nardone et al. 2010), and reproduction (De Rensis and Scaramuzzi 2003), notably of genetically superior merit cows and animal production parameters like growth rate, feed conversion ratio, etc. Farm management strategies have typically focused on improving efficiency, reducing expenses, and maximizing profit. However, environmental, animal welfare, and food safety problems must now be considered as well. Because animal farming techniques are highly diversified, the related GHG emissions per kg produced vary significantly in different regions. Within the dairy business, both milk processors and farming organizations have realized the necessity to evaluate their influence on the environment regarding GHGs. Certain studies have been undertaken to estimate emission factors for enteric CH_4 emissions and dung CH_4 and N_2O emissions by changing the ration composition. However, we have no clue how much GHG is emitted when cattle and buffalo herds produce milk. The LCA technique assesses the environmental implications of GHG emissions and emissions intensity (Ei) for milk production with system boundaries (Guinée and Lindeijer 2002). This is frequently referred to as a "cradle-to-grave" approach. The emissions are computed using whole-farm GHG models with a yearly time-step or country-specific default emission parameters based on IPCC methodology (Van Middelaar et al. 2019). The LCA methodology aids in determining the strengths and weaknesses of livestock production systems in any region or area of study.

12.2 Life Cycle Assessment (LCA) Methodology

The goal of assessing the environmental effects of agricultural goods is to determine their impact on environmental sustainability (Dalgaard et al. 2006), particularly in terms of food consumption patterns (Wallgren and Höjer 2009). According to Lal (2004), the system's sustainability can be assessed using inputs and outputs and their conversion to CO_2. Monni et al. (2007) stated that there are several inaccuracies in measuring GHG emissions. This error occurs because agricultural emissions are governed by complicated biological processes involving a range of factors.

LCA and energy analysis or ecological footprint (Thomassen and de Boer 2005) are some of the methodologies that can examine the environmental implications of agricultural activities (Finnveden et al. 2009). The LCA technique can be summarized as an evaluation including all inputs, outputs, and potential effects to the environment throughout a life cycle from the cradle to the grave, e.g., from the extraction of raw material through materials processing, production, supply, use, maintenance and repair, and disposal or recycling (Jørgensen et al. 2008). It is an ISO-mandated way of communicating a product's environmental impact (ISO 14040, 2006).

LCA is mainly an environmental accounting method that totals GHG emissions to the environment across a product's entire production chain (e.g., milk or beef), taking into account both direct (e.g., manure/enteric methane emissions from cattle) and indirect emission sources that are GHG emissions linked with fertilizer used for fodder production, energy used for feed and fodder processing, etc. (Rotz and Veith 2013). Much research has been undertaken on industrial materials and manufactured products, but the tendency is now shifting to animal industries, particularly the dairy and beef sectors. Identifying potential opportunities for enhancing environmental performance within a production system is one of the most valuable outcomes of conducting LCA for animal production.

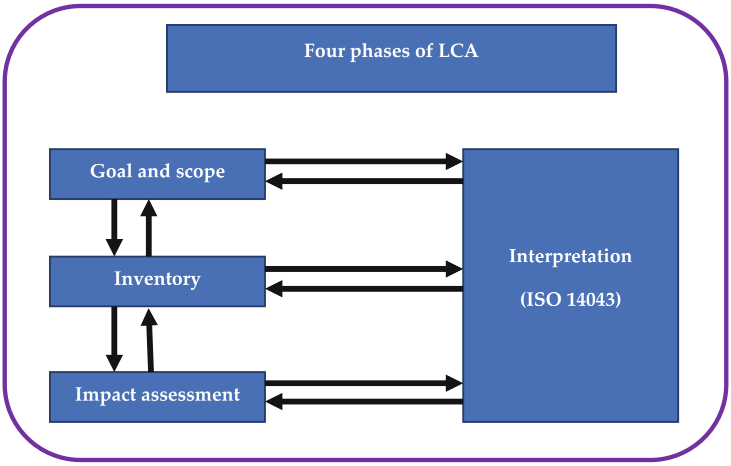

Fig. 12.1 Four phases of life cycle assessments (LCA) (Beauchemin and McGeough 2013)

The LCA approach is typically used to assess climate, water pollution, and air pollution impact categories. On the other hand, impacts such as biodiversity and pesticide toxicity are rarely assessed due to methodological issues (Thomassen et al. 2008). The LCA study consists of four phases are outlined by Beauchemin and McGeough (2013) as definition of goal and scope, life cycle inventory analysis, impact assessment, and interpretation (Fig. 12.1).

12.2.1 Goal and Scope

The purpose and scope of study will determine the experimental results and its legitimacy (Kočí 2009. This phase establishes the study's goal and the functional unit and system boundaries (Fig. 12.2). The functional unit represents the system's output and serves as a reference point for expressing environmental consequences. The system boundary defines all aspects of the system that are included or excluded from the LCA study (Crosson et al. 2011). Different system boundaries, functional units, and farming systems were used during the LCA study, such as up to retailer and up to farm gate. 1 kg milk, 1 kg processed milk, 1 kg FCM, and 1 kg FPCM are examples of functional units. Various agricultural methods are available, such as conventional farms, organic farms, small-scale dairy farms, and organized farms, because most GHG emissions from meat and milk are produced until the product leaves the farm gate. Most LCAs use a system boundary that ends at the farm gate (also known as partial LCA) (Beauchemin and McGeough 2013). When the LCA border extends beyond the farm gate, end-product operations such as food processing, transportation, and post-harvest handling are included (Caffrey and Veal 2013). Direct emissions from animal production units via enteric fermentation

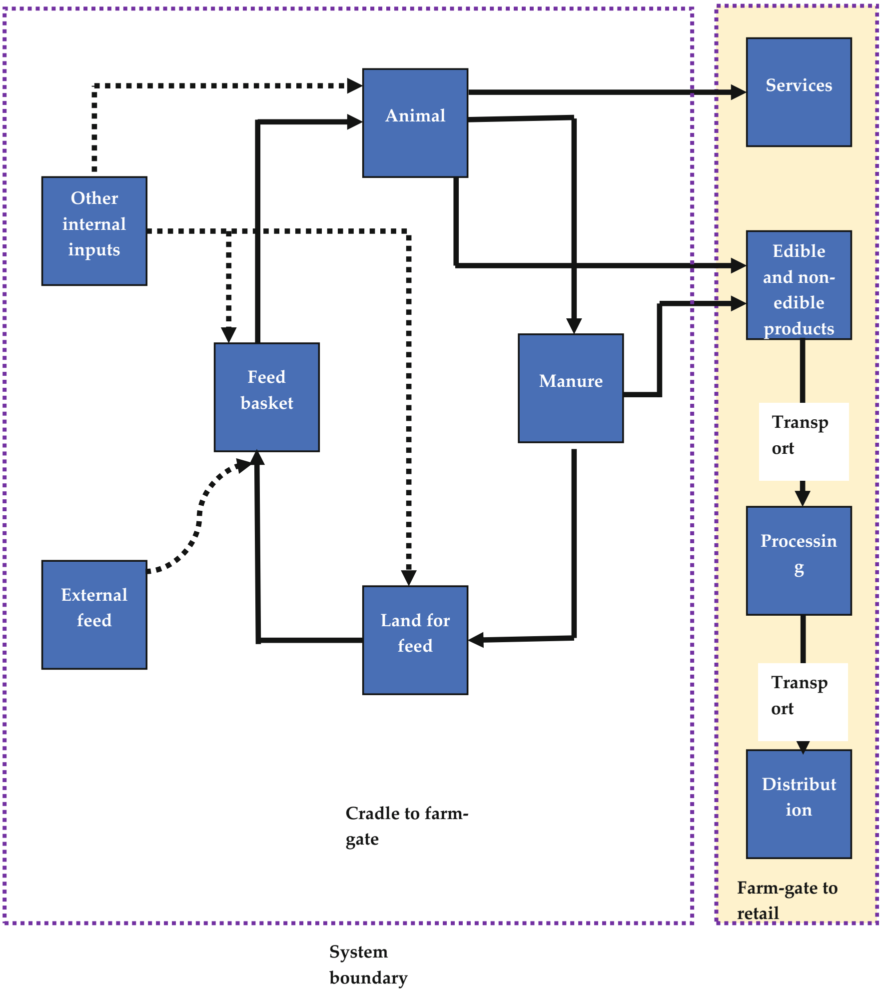

Fig. 12.2 System boundary defines all areas which are included or excluded in the LCA study

and manure management are included in the LCA study, as well as indirect emissions from fertilizer used for fodder production (N_2O), electricity used for feed processing (CO_2), diesel used for feed transportation (CO_2), and other sources.

12.2.2 Life Cycle Inventory Analysis (LCI)

Inventory analysis contains information such as the number of animals in a unit, the type of animals, and the feed and fodder requirements of the animals, among other things. All input and output are collected for all processes required for the LCA research, and calculations are performed using key EFs or default factors. IPCC (2006) provides certain EFs that are used in the LCA study.

12.2.3 Impact Assessment

Emissions are categorized and multiplied by their characterization factor in this phase. Total GHG emissions comprise CO_2, CH_4, and N_2O from feed production (cultivation, processing, and transport), CH_4 from enteric fermentation, and CH_4 and N_2O from manure management. After considering the global warming potential, the total amount of GHG emissions was converted to CO_2 equivalents.

12.2.4 Interpretation

Based on the original objective, interpretation gives suggestions and conclusions based on the LCA study.

12.3 Attributional and Consequential Approaches of LCA

ALCA (attributional life cycle assessment) provides data on the impact of the procedures used to manufacture, consume, and dispose of a product. However, it does not consider indirect effects caused by changes in the product's output (Thomassen et al. 2008). ALCA is beneficial for consumption-based carbon accounting because it provides information on the average product unit (Fig. 12.3). CLCA (consequential life cycle assessment) gives information on the implications of changes in a product's output, consumption, and disposal, encompassing repercussions both inside and outside the product's life cycle. CLCA attempts to advise policymakers on the broader consequences of policies aimed at changing production levels by modeling the causal relationship that arises from adjusting the product's output (Brander et al. 2008).

12.4 Calculation of Emission Factors for LCA

Direct emissions from animal production units via enteric fermentation and manure management are included in the LCA study, as well as indirect emissions from fertilizer used for fodder production (N_2O), diesel used for feed transportation (CO_2), electricity used for feed processing (CO_2), and other sources. Chapters 10

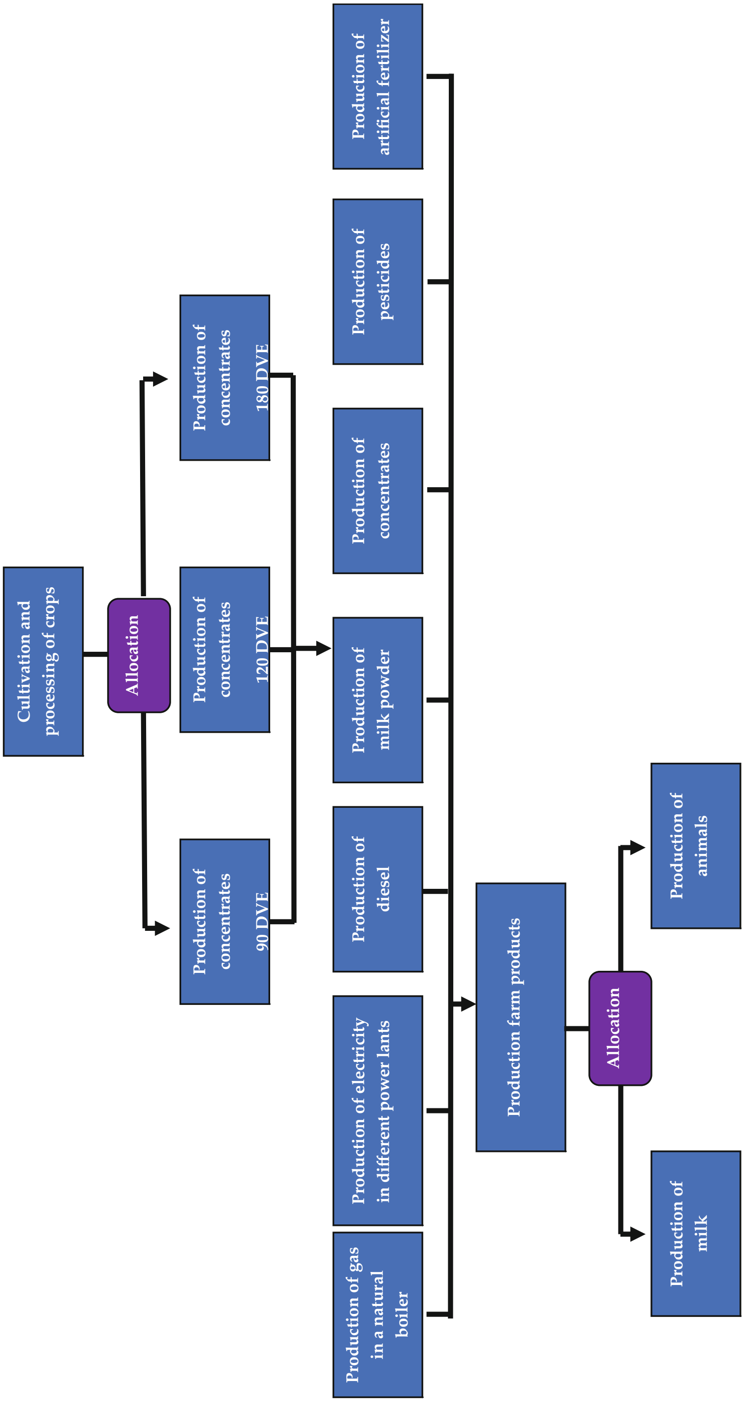

Fig. 12.3 Flow diagram of attributional approach of LCA (Thomassen et al. 2008)

and 11 are used to calculate emissions based on IPCC (2007) guidelines. A combination of IPCC (2006) Tier 1 and Tier 2 methodologies and emission factors (EFs) were used to estimate emissions. Despite the existence of country-specific EFs, the evaluation was conducted using a unified approach to guarantee that the results were consistent and comparable across different regions and farming systems. The characterization of livestock populations and the calculation of emissions linked to enteric fermentation, manure management, and storage were done using IPCC Tier 2 methodology. When data was scarce, such as estimating carbon stocks from LUC and N_2O emissions from feed production, the Tier 1 technique was used.

12.5 Greenhouse Gas Emissions from Animal Production Unit

12.5.1 Emission Factors for Enteric Fermentation

Enteric fermentation emissions always account for most overall GHG emissions for milk production on a single farm, making livestock one of the significant contributors to GHG emissions. These emissions cannot be totally avoided due to the dairy animals' natural digestion process, and they are influenced by the number and proportion of non-milking animals (calves, heifers, and bulls) on the farm. In ruminants, feed digestion is a two-stage process: (1) enzymatic degradation of feed sources in the rumen, which releases a variety of monomers such as sugars, amino acids, glycerol, and fatty acids, and (2) fermentation of those compounds by rumen microbes like bacteria, methanogenic archaea, protozoa, and fungi (Hristov et al. 2013). The reticulo-rumen-omasal complex (forestomach) environment is intrinsically tightly regulated for pre-gastric fermentation of cellulose-rich meals (redox potential of −300 to −350 mV; 38–42 and pH 6–7). CO_2 is expelled from the rumen's head region and lost through eructation or supplied to the lungs and inhaled by the animal, whereas VFAs are transmitted through the rumen and omasal walls and utilized by the animal (Hill et al. 2016). H_2 is cleared from the body through VFA production or, more commonly, conversion to CH_4. Methanogenic archaea help to facilitate this latter process. They use metabolic hydrogen to aid in reducing and reoxidation of rumen bacteria, resulting in ATP production (McNeely et al. 2010).

There are three distinct pathways for methanogenesis:

1. Acetoclastic methanogenesis—It occurs in the methanosarcinales (e.g., methanosarcina and methanosaeta).

$$CH_3\,COOH \rightarrow CO_2 + CH_4$$

2. Methylotrophic methanogenesis—In this pathway, methanol or methylamines serve as substrates.

$$CH_3\,OH + H_2 \rightarrow CH_4 + H_2O.$$

$$4CH_3\,OH \rightarrow 3CH_4 + CO_2 + 2\,H_2O.$$

$$CH_3\,NH_2 + H_2 \rightarrow CH_4 + NH_3$$

3. Hydrogenotrophic methanogenesis—It uses H_2 for the reduction of CO_2 (or CO or formate).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O.$$

Enteric methane emissions mainly depend upon the type of feed, level of production by the animals, and its physiological state. A higher proportion of non-milk-producing animals (calves, heifers, and bulls) will lead to higher GHG emissions per liter of milk produced. Overall, livestock emits 14.5% of global GHGs, including CH_4 through enteric fermentation and manure management, as well as N_2O from manure and fertilizer application (FAO 2015). As part of their natural digestive processes, enteric fermentation by ruminants is responsible for about 37% of anthropogenic CH_4. Malik and Singhal (2009) found that adding 30% of lucerne fodder (II cut) to the treatment ration, which substituted wheat straw in the control ration, reduced enteric CH_4 emission by 32% in male Murrah buffalo calves. According to Jain et al. (2011), CH_4 emission per kg DMI was reduced by 20% after supplementation with the herbal mixture (1000 ppm), with CH_4 emission levels of 22.67 g/kg DMI for the control group and 18.46 g/kg DMI for the experimental group, respectively. Garg et al. (2012) observed an increase in milk yield (kg/day) of 0.46 and a reduction in CH_4 emission (g/day) of 13.30% after feeding a balanced ration to lactating buffaloes. According to Odongo et al. (2007), long-term effects of monensin supplementation (24 mg of Rumensin Premix/kg dry matter) in lactating dairy cattle were observed to reduce CH_4 production by 7% (g/d) and 9% (g/kg B Wt.).

Tier 2 approach (IPCC 2006) can be used to assess enteric CH_4 emissions, which involves calculating CH_4 as a function of gross energy intake:

$$\text{kg } CH_4/\text{day} = (GE \times Ym)/55.65$$

GE = gross energy intake
Ym = CH_4 conversion rate, which is the fraction of gross energy in the feed that is transformed to CH_4 (%)

Some other equations used to predict enteric methane emission in LCA studies are given in Table 12.1.

Table 12.1 Enteric methane prediction equations commonly used in life cycle assessments (LCA)

Model	Enteric methane (CH_4) emissions prediction equation	References
IPCC Tier 2	CH_4/head/yr. (kg) = (GE[a] × Ym[b] × 365 days/yr)/(55.65 MJ/kg CH_4)[b]	Casey and Holden (2005); Nguyen et al. (2010); Pelletier et al. (2010); Beauchemin et al. (2010)
Shibata et al., (1993)	CH_4 (L/d) = −17.766 + 42.793 × (kg DMI[c]/d) − 0.849 × (kg DMI/d)^2	Ogino et al. (2007)
Mills et al. (2003)	CH_4/head/day (kg) = [Emax[d] – Emax (−c[e] × MEI[f])] × FkgCH_4	Stackhouse-Lawson et al. (2012)
Blaxter and Clapperton (1965)	CH_4/head/day (kg) = 1.3 + 0.112DMDijkl[g] + Lijkl[h] (2.37 − 0.050DMDijkl)	Peters et al. (2010)
Moe and Tyrrell (1979)	CH_4/head/day (MJ) = 3.406 + 0.510 NFC[i] + 1.736 HC[j] + 2.648 C[k]	Capper (2011); Peters et al. (2010), feedlot emissions

[a] GE = gross energy intake (MJ/head/day)
[b] 55.65 (MJ/kg CH_4) is the percentage of gross dietary energy lost as methane
[c] Dry matter intake
[d] Emax = maximum possible emission, 45.98 MJ CH_4/head/day
[e] c = −0.0011 · [Starch/ADF] + 0.0045, Where: Starch = starch content of diet, ADF = acid detergent fiber content of diet
[f] MEI = metabolizable energy intake, MJ/head/day
[g] DMDijkl = digestibility of feed (expressed as a %)
[h] Lijkl = feed intake relative to that needed for maintenance
[i] NFC = nonfiber carbohydrate (kg/d)
[j] HC = hemicellulose (kg/d)
[k] C = cellulose (kg/d)
[l] Ym = methane conversion factor, percent of gross energy in feed converted to methane
[m] FkgCH_4 = conversion of MJ to kg of CH_4, 0.018 kg CH4/MJ

12.5.2 Emission Factors for Manure Management

This section explains how to calculate the amount of CH_4 produced by manure storage and treatment, as well as manure deposited on pasture. Manure management is a critical component of farm nutrient budgeting and is closely linked to fertilizer inputs. The term “manure” is used in this context to refer to both dung and urine (i.e., solids and liquids) produced by animals. CH_4 is produced by the anaerobic breakdown of manure during storage and treatment. Livestock manure is primarily made up of organic matter and water, which is digested by anaerobic and facultative bacteria under anaerobic conditions to produce CH_4, CO_2, and stabilized organic material as an end product. Manure’s capacity to produce CH_4 is determined by its composition, climate (temperature and rainfall), manure management system (liquid vs. dry), pH levels, water content, and nutrient availability. The quantity of manure generated impacts CH_4 generation from livestock dung, which varies by animal type and is proportionate to the animal’s weight and feed intake (Kariyapperuma et al. 2018). The volatile solids fraction of manure is the part that

Table 12.2 Tentative default values for N_2O emission factors from animal waste per animal waste management system (kg N_2O—N/kg nitrogen excreted)

Animal waste management system	Emission factor (EF)
Anaerobic lagoons	0.001 (<0.002)
Liquid systems	0.001 (<0.001)
Daily spread[a]	0.0 (no range)
Solid storage and drylot[a]	0.02 (0.005–0.03)
Pasture range and paddock (grazing)[b]	0.02 (0.005–0.03)
Used as fuel[c]	Not Applicable
Other systems[b]	0.005

[a] Considered to be a part of direct soil emissions from agricultural fields after spreading
[b] Considered to be a part of direct soil emissions from animal production
[c] Considered to be a part of emissions from energy

can produce CH_4 (Leitner et al. 2021; Jun et al. 2000). When manure is kept or processed as a liquid, for example, in lagoons, ponds, tanks, or pits, it decomposes anaerobically and creates a significant amount of CH_4. However, when manure is treated as a solid (e.g., in stacks or piles) or dispersed on rangelands and pastures, it decomposes under more aerobic circumstances, producing less CH_4. The nitrification-denitrification of nitrogen in livestock manure produces N_2O, which is also a greenhouse gas. Ammonia is the most common type of nitrogen in manure (NH_3). Nitrification transforms ammonia to nitrate in an aerobic environment, whereas denitrification converts nitrate to N_2O in an anaerobic one. The amount of N_2O released is determined by the waste management system, duration, temperature, and pH (Signor and Cerri 2013).

Tier 2 technique (IPCC 2006) is used to determine CH_4 emissions from manure storage as a function of manure volatile solids (VS) and mean air temperature:

$$\text{kg CH}_4/\text{day} = \text{VS manure}/\text{day B}_\text{O} \times 0.67 \times \text{KMCF factor}$$

where VS manure denotes the daily volatile solids of manure, B_O denotes the maximal CH_4 generating capacity (m^3/kg VS) for manure produced (0.24 m^3/kg VS), and KMCF is the CH_4 emission capacity of manures.

Estimates of kilograms of nitrous oxide nitrogen produced per kilogram of nitrogen expelled are known as nitrous oxide emission factors. These factors vary with waste management systems, as shown in Table 12.2.

12.5.3 *Emission Factors for Fertilizers Used for Fodder Production*

India stands in the second position as a consumer of fertilizer in the world next to China. Fertilizer is an essential part of sustainable agriculture, and currently, mineral nitrogen fertilizers are the only way to feed roughly half of the world's population

(Erisman et al. 2008). Plant growth and yield depend on sufficient amounts of plant nutrients and their proper balance. Mineral fertilizers provide essential nutrients for sustainable agriculture production. They fill that gap between the nutrient requirements for optimal crop production and the nutrients available from soil and organic sources. Fertilizers are substances that provide essential nutrients to plants, especially significant nutrient nitrogen, potassium, and phosphorus which are deficient in soils. These nutrient applications through fertilizer optimize crop and fodder production.

Mineral fertilizer production and use in agriculture should be considered an essential aspect of global food production and as part of the solution to climate change issues. Mineral fertilizers enable the targeted application of particular nutrients at calibrated rates, resulting in balanced crop nutrition and improved soil fertility. A lack of nutrients, in particular, lowers crop yields in many developing countries (Sanchez et al. 2007). As a result, increasing fertilizer inputs (+37%) is critical in improving agricultural output in future crop production. On the other hand, the fertilizer business uses energy and releases CO_2 and other GHGs during manufacture, transportation, and consumption, resulting in climate change. Agricultural soil is a significant producer of N_2O, a GHG that also adds to stratospheric ozone damage. As with all models, a set of guidelines is established before any calculations are done. This is the case with the IPCC's 1996 and 2000 guidelines (also known as the Intergovernmental Panel on Climate Change 1996, 2000), which specify a default fertilizer-induced N_2O EF of 1.25% of net N input (on the basis of unvolatilized fraction of the applied N) for direct emission from agricultural soil and a background N_2O emission of 1 kg N ha^{-1} $year^{-1}$ (IPCC 1997). Here EF is represented as a percentage of the N applied and is expressed as the emission from fertilized plots less the background emission (emission from a zero-N control plot). Sweden's country-specific EF ranges between 1 and 1.5%, depending on the quantities of various fertilizers sold (Swedish Environmental Protection Agency 2006). EFs in the Netherlands range from 1% for chemical fertilizers to 2% for manure (Netherlands Environmental Assessment Agency 2006). In the future, research will be required into the impact of fertilizers on climate change and how new production technology and optimal fertilizer application lead to significant improvements in agricultural production's GHG balance. Country-specific carbon footprints can be used to calculate the contribution of fertilizer production and for estimating carbon footprint.

12.5.4 Emission Factors for Electricity Generation and Utilization

Electricity consumption plays a vital role in running any type of production system organization as well, as it acts as a most prominent source of emissions; therefore, it is crucial to measure these emissions as accurately as possible. International Energy

Agency (IEA 2010) published new country-specific emissions factors (composite factors) for calculating emissions from electricity consumption for the majority of countries and accounts GHG emissions for electricity generation as well as other GHG emissions for transmission and distribution losses for consumed electricity (kWh) (Brander et al. 2008). Emissions from electricity consumption are calculated by multiplying the amount of power consumed by an EF. These emission factors for each kWh consumed electricity are critical in life cycle assessments (Herrera et al. 2015). Electricity sources emit 0.0005883 metric tons CO_2 per kWh or 1.297 lbs. CO_2 per kWh on average. It is necessary to keep track of the amount of electricity used in various activities related to milk production (Mary Lissy 2012). Brander et al. (2008) reported that the electricity emission factor for India was 1.33 kg CO_2/kWh. In India, total GHG emissions from electricity production were 719.31 million tons CO_2 equivalent in 2007. This accounts for 65.4% of total CO_2 eq. emitted by the energy industry. Due to energy utilization in those activities, the agriculture/fisheries sector emitted 33.66 million tons of CO_2 eq (INCCA 2010). According to Electricity GHG Inventory Report 2009–2010, the average value of EF for electricity consumption is 1.19 CO_2 eq. /kWh in India.

12.5.5 Emission Factors for Diesel Consumption Used for Transport

Fossil fuels power transportation, and the amount of carbon dioxide generated by each fuel differs. Tractors are used in fields and livestock farms for feed and fodder transportation in the livestock production process. The tractor engine burns gasoline, emitting CO_2 in varying amounts depending on the fuel and contributing to GHG emissions. However, high-speed diesel (HSD) is utilized for transportation, energy generation via diesel generators, and running irrigation pumps in the agricultural sector. Depending on the fuel type, consumption, driving lengths, and vehicle type and condition, the car's engine burns gasoline and emits a particular quantity of CO_2. One liter of petrol and diesel produces 2.3 kilograms and 2.7 kg of CO_2, respectively (Telecom regulatory authority of India 2011). According to INCCA (2010), India's transportation industry emitted 142.04 million tons of CO2 equivalent from road transport, aircraft, railways, and navigation in 2007, with road transport accounting for 87% of the total CO_2 equivalent emissions. Transportation-related fossil fuels are responsible for almost 13% of worldwide GHG emissions. Details of the number of vehicles, kind of vehicle, and the fuel used should be documented to compute greenhouse gas emissions for milk production through transportation. This information gives us an idea of how much petrol or diesel is used as fuel by these vehicles. Compared to diesel, the amount of carbon dioxide emitted by petrol is lower (Mary Lissy 2012).

12.5.6 LCA of Greenhouse Gas Emissions for Milk Production

Several studies have been conducted using the LCA method to estimate greenhouse gas emissions for milk production in various countries. According to Casey and Holdon (2005), the average GHG emission at the farm gate is around 1.50 kg CO2-eq. Each kilogram of ECM per year and 1.3 kg of CO2 equivalent with economic distribution between meat and milk. Enteric fermentation accounted for 49% of total emissions, followed by fertilizer (21%), concentrate feed (13%), dung management (11%), and electricity and fuel consumption (5%) per kg per year. According to Capper et al. (2009), the production system substantially impacts milk carbon footprint, revealing that technological developments between 1944 and 2007 led to a reduction in CO_2eq per kg of milk from 3.66 to 1.35 kg. According to FAO (2010), the average GHG emission at the farm gate is around 2.4 kg CO_2eq per kg of FPCM, with significant regional variations: 1 kg CO_2eq in North America to 7.5 kg CO_2eq in South Africa. The overall greenhouse gas emissions (on a CO_2 equivalent basis from enteric fermentation, manure management, and feed production at the farm gate) for milk production under the smallholder dairy system in Anand district were 2.2 kg CO_2 equivalent/kg FPCM, which decreased to 1.7 kg CO_2 equivalent/kg FPCM when milk, manure, finance, and insurance were considered as economic functions of the smallholder dairy system, according to Garg et al. (2016).

12.5.7 Factors Affecting Carbon Footprint in Livestock Products

Compared to other foods, livestock products have a high carbon footprint (CF); thus, it is crucial to focus on lowering it. Agricultural emissions account for 70–90% of the entire chain's emissions. Hence the agricultural stage is essential in evaluating the carbon footprint of most food commodities. With the growing world's population, a considerable demand for livestock products can be expected in the coming decades. This means that a more significant amount of animal protein must be produced with fewer resources (land, water, energy) and reduced CF. By enhancing productivity, the livestock sector can reduce resource use and waste generation per food unit (i.e., animal protein output per unit input). CF is exacerbated by emissions from feed intake and manure handling and the type of the land required to produce the feed. As a result, the following are the most critical mitigating strategies:

- At the system level, improved feed efficiency.
- Utilize manure for bio-energy production.
- Ascertain if the manure produced is a viable alternative to synthetic fertilizer.
- Increased carbon sequestration in the soil vs. carbon emissions.

12.6 Conclusion

The livestock sector has a significant impact on the environment due to pollution emissions into the air, water, and soil and the utilization of limited resources. The current climate change trend poses a threat to the product efficacy and welfare of livestock worldwide. LCA is a holistic method for testing the environmental implications of products, methods, or activities across the course of an animal's whole life cycle. When GHG emissions from dairy farms receive much attention, CF could be utilized for benchmarking by comparing GHG emissions from cattle from various nations or production practices. Moreover, it might also assess how a farm, a region, or a state has improved due to technological advancements or political strategies. Moreover, it also gives us information on the extent to which inventories alter emission levels, allowing us to understand how and to what extent each of our actions affects environmental change. In the current environment of rising CO2 levels in the atmosphere, the scope of such research is highly relevant. LCA research on milk would aid in identifying the elements that lead to the excessive greenhouse gas emissions associated with milk production and propose solutions for a cleaner, greener tomorrow.

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Chapter 13
Cow Dung as a Renewable Source of a Domestic Fuel

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Abstract Instead of relying on fossil fuels, the globe is increasingly exploring other energy sources. Using fossil fuels, which is into greenhouse gas emissions and rapidly depleting, the energy is environmentally friendly and renewable. The massive volumes of this waste created each year necessitate the development of innovative approaches and methods for managing the massive amounts of organic waste discharged unlawfully into mineral deposits. Cow dung is among the most prevalent organic wastes in the world, posing a threat to our environment since it emits greenhouse gases, causes odours, and depletes freshwater resources. Cow dung, on other hand, has a variety of characteristics that make it a viable source of renewable energy and an organic natural soil supplement. In terms of the volume of cow dung generated, all researchers have a major chance and opportunity to research the waste. Anaerobic digestion is among the most effective enzymatic techniques for managing cow dung. It is seen to be an appealing strategy, particularly in context of sustainable energy such as biofertilisers and biogas. From different points of view, the present article examines cow dung as a coating molecule in the composting process.

Keywords Anaerobic co-digestion · Biogas · Biodegradable organic based · Geothermalnic

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_13

13.1 Introduction

Economic advancement, social development, human well-being, and the improvement of quality of life all require energy. Agriculture, manufacturing, transportation, commerce, and home utilise energy. As a result, total energy consumption has been steadily growing across the country. As a result of the rising energy usage, the country has become more dependent on fossil fuels such as coal, oil, and gas. The management of all sorts of trash, as well as the energy crisis, are two of the world's most pressing issues today. Increased applicatory utilisation of natural fuels such as fossil fuels negatively influences the ecosystem on both a local and global scale. As a direct result of population expansion, urbanisation, and industrialisation, the globe will unavoidably face crises of fossil fuel shortages and environmental deterioration (Sorathia et al. 2012).

When we discuss renewable energy, animal dung is a resource that may be used as an alternative to industrial fertilisers and increase soil fertility. Natural waste, on the other hand, may form water, smell, and depletion of soil, if the emission of greenhouse gas is not managed well (Burg et al. 2018). When utilised as a co-substrate in anaerobic co-digestion process, cow dung offers numerous advantages and benefits, including boosting methane production and balancing low substrate characteristics as carbon and nitrogen ratio, pH, and presence of minerals (Zhao et al. 2018).

Total yearly quantity of animal dung generated, with sewage sludge topping the list with the yield of 7200 tonnes per year, after which cow manure and pig manure are ranked with yield value 6500 tonnes in a year (Ruiz et al. 2018). The amount of manure produced each year is around 2×10^6 which only includes dry weight; with cow dung accounting for 76% or in rest, 10% consists of horse manure, 6% for pig manure, 2% for poultry. From the above-mentioned figures, this is obvious that there are a large number of animal wastes that can be converted into renewable energy rather than being thrown (Burg et al. 2018).

Biogas is a sustainable energy source produced by oxygen-independent digestion of organic biodegradable raw matter which includes wastes from industry and municipal as well as agricultural and animal litters. Biogas contains a high concentration of methane gas (40–70%), which may be improved to natural gas quality (75–99% methane content). The improved biogas can be utilised as a transportation fuel or pumped into a natural gas system (Mittal et al. 2018).

Apart from providing energy and manure, anaerobic digestion of biodegradable organic wastes has several social and environmental advantages. Because of its characteristics, cow dung is an ideal substrate for anaerobic digestion. Although land spreading is the most prevalent application of cow dung, there may be a leftover that anaerobic digestion could refine. After the procedure, this technique can provide a sufficient digest that is rich in key plant nutrients (C/K/N/P). The microbial population, on the other hand, eats soluble organic materials. As a result, in anaerobic digestion, solubility and humidity percentage are critical (Li et al. 2019; Siddique and Wahid 2018). The benefits of anaerobic digestion technology are numerous, including economic, health, environmental, and social. Obtained biogas can be utilised as a source of electricity and heat and considered pure based on usage of energy and gas emission by combustion. Digestate, a side product obtained along this process, is likewise high in nutrients that may be used in agriculture (Li et al. 2019; Mengistu et al. 2015).

In India in 2010, a total of 5800 biogas plants were operational, generating 2300 MW of energy. The USA generates 57.1 MW of power from 160 biogas facilities. Biogas output in Europe is at 10.9 Mt., with Germany leading with 61% of all renewable energy production, followed by the UK with 16.5%. By 2020, Germany is anticipated to have 43,000 biogas plants. China is also one of the leading nations in terms of biogas plant implementation, with over 4700 plants of biogas and a rate for the production of four billion m^3 of biogas in a single year. There are over 14,000 residential biogas digesters that have been installed in developing nations such as Ethiopia, indicating that the globe is now seeking for an alternative source of energy that is also environmentally benign, such as anaerobic digestion (Mengistu et al. 2015).

13.2 Sources of Renewable Energy

Renewable energy sources (RES) currently meet 14% of total global energy demand. In 1998, renewable energy sources accounted for 2% of global energy consumption, with modern biomass accounting for seven exajoules and all other renewables accounting for two exajoules (Goldemberg et al. 2000). Instances for natural and renewable resources are geothermal, biomass solar, hydropower, wind, and marine energy. Renewable energy sources are the most common. Renewable energies such as nuclear energy and hydrogen are clean or limitless sources of energy. The most significant advantage of renewable energy sources is the reduction of pollutants. Production of RES from mechanical, thermal natural and growth processes that occur repeatedly throughout lives and can be counted on to supply predictable amounts of energy when needed. Water and wind power, for example, are unlikely to have delivered the same rapid growth in industrial productivity as fossil fuels did. Fossil fuel burning accounts for around 98% of carbon emissions. Avoiding the usage of fossil fuels would result in a significant reduction in the quantity of CO_2 release, as well as lower liberation of pollutants. Moreover, the accessibility, price of free carbon, and carbon-diminishing technology like energy-efficient and conservation of energy tools account for a large portion of the difference in cost estimates for decreasing carbon emissions. This could be accomplished by consuming less energy overall or by utilising alternative energy sources.

13.2.1 Biomass

Biomass is a pleasing renewable fuel to use in boilers to augment coal burning. Forest debris, alfalfa stems, periodic herbaceous crops, and additional types of manure, gas, short-rotation woody crops, and wastewater treatment gas are among the biomass fuels being explored for co-firing (Fig. 13.1). At now, solid waste is a low-cost fuel only when it is obtained along the process of a highly significant activity/product. Solid wastes are a renewable and long-lasting resource. In emerging nations, biomass accounts for 35% of primary energy consumption, bringing the global total to 14% of primary energy consumption. Biomass has the ability to provide a cost-effective and long-term energy supply while also assisting governments in reaching their greenhouse gas reduction objectives in the future. Biomass energy now accounts for around 14% of global final energy consumption, a larger percentage than coal (12%) and equivalent to gas (15%) and electricity (15%) (14%). For many poor nations, biomass is the primary source of energy, and the majority of it is non-commercial. As a result, gathering trustworthy biomass energy statistics is extremely challenging. However, excellent data is required for assessing trends and consumption patterns, forecasting future trends, and developing cohesive plans (Demirbas 2005).

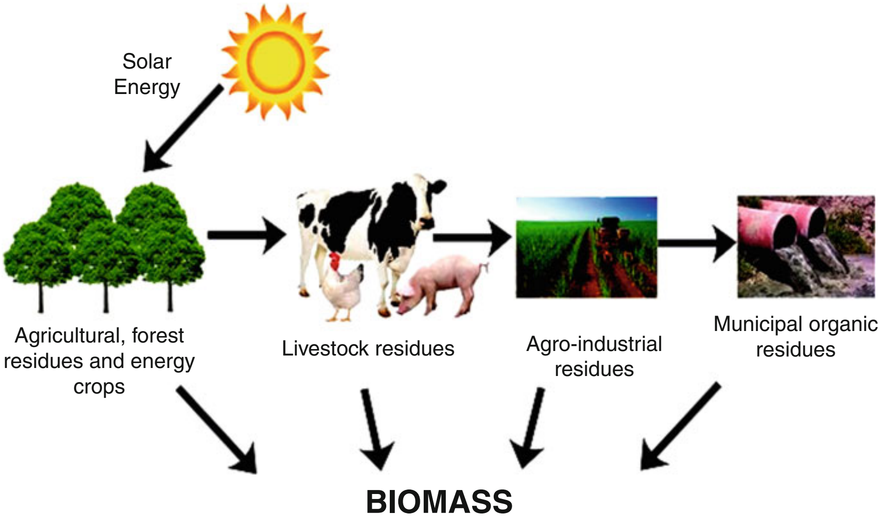

Fig. 13.1 Various biomass sources

13.2.2 Hydropower

River and stream, water may be caught and converted into hydropower, commonly known as hydroelectric power. Huge-scale hydropower generates roughly a fourth of the world's entire electricity, whole electricity of Norway, and more than 40% of the electricity exhausted by developing nations. The world's theoretically viable immense scale of hydro potential is expected to be around 2200 GW from which approximately 25% is presently utilised. Micro hydropower systems (MHP) with powers below 100 kW and small hydropower systems (SHP) with outputs between 101 kW and 1 MW are the two types of small hydropower plants. Huge-scale hydropower provides 20% electricity on global scale. Although there is still a lot of promise in developing nations, big hydropower projects might encounter financial, environmental, and social challenges. These systems provide low-cost energy generation, which is used in a number of poor nations across the world (Fig. 13.2).

13.2.3 Geothermal Energy

The geothermal energy usage includes spatial heating and domestic heating, CO_2 and dry ice production processes, heat pumps, heat from the greenhouse, therapeutic baths (balneology), swimming, industrial operation, and power production. Bathing, swimming, and balneology (42%) are the most common direct applications, followed by space heating with 35%, greenhouses (9%), pisciculture (6%), and industry (6%) (Fridleifsson 2001). Electricity production, direct consumption, room heating, heat pumps, heat cause of greenhouse effect, and industrial use are

Fig. 13.2 Hydropower systems

Fig. 13.3 Geothermal energy systems

all examples of geothermal energy applications. Geothermal steam gas is used to generate electricity in 21 nations throughout the globe. To create heat, many nations use low-temperature geothermal energy, with a capacity expected as of 10,000 MW thermal (Demirbas 2005) (Fig. 13.3).

13.2.4 Solar Energy

Solar systems in houses, SPV (solar photovoltaic systems), SWH (solar water heating) systems, and solar-based dryers and cookers are all examples of solar energy systems. A family or a small community installs and maintains these systems. A PV system with a capacity of 40 watts fully is referred to as a solar household system. A family or a small community installs and maintains these systems. Solar energy can be used to break up the water molecules into hydrogen and oxygen, which is an interesting way to convert energy from solar to chemical (Fig. 13.4).

13.2.5 Wind Energy

For millennia, windmills have been used to grind wheat or pump water using renewable energy from the wind. Large wind turbines have been built in recent years to generate power. This non-polluting form of energy is widely available and non-polluting. The efficiency of wind turbines is improving. The price of the power they produce is decreasing. Wind power is also promising in coastal and other windy areas. Wind energy is no more a substitute of energy source by any means. Wind energy has geographical and meteorological constraints, and issues associated with installing turbines could be environmental or political (Demirbas 2005) (Fig. 13.5).

Fig. 13.4 Solar energy systems

Fig. 13.5 Wind energy systems

13.3 Biogas Production from Cow Dung

Biogas is the only biomass-derived contemporary energy carrier for home applications with which there is extensive experience. Biogas is a clean-burning methane-rich fuel gas generated by anaerobic digestion (bacterial activity in a tank without

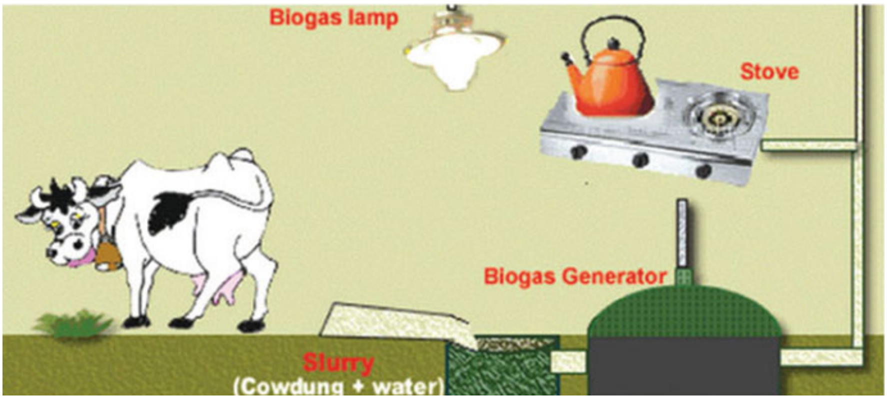

Fig. 13.6 Production of biogas from cow dung

air) of appropriate biomass feedstocks. Biogas may be made from cow dung and animal wastes, as well as some agricultural leftovers with a lot of effort. Despite the fact that these feedstocks are commonly used directly as cooking fuel, they are not favoured fuels in most places and are only utilised when wood is unavailable. Biogas systems have several advantages. The digester effluent is typically an excellent fertiliser, and biogas facilities can provide significant sanitation services if they are connected to latrines. It is straightforward and relatively efficient to utilise the gas directly in traditional low-pressure gas burners for cooking and other thermal home chores. When utilised in mantle lamps, biogas may also produce lighting (Fig. 13.6).

The huge amount of organic waste is created each year in Bangladesh's rural areas, with cow dung topping the list at 102.6 million tonnes per year. They stated that this organic waste has a high economic worth in terms of producing green powers like from biogas and bio-fertilisers in aim to get commercial, environmental, and well-being balance. The authors used a method known as life cycle analysis to link several energy techways available to handle natural waste created year on year to fulfil energy demands of agricultural regions (LCA). To analyse the parameters impacting the efficiency of this system, data was collected from 20 biogas plants and 20 solar photovoltaic panels. It was pointed out that chicken waste produces more biogas than cow dung and that solar systems and natural gas plants employing cow dung and other wastes are sustaining, according to the life cycle analysis (LCA) (Ali et al. 2019).

Anaerobic co-digestion of cow dung and pear waste complete in a totally mixed stir-ring tank reactor under mesophilic and anaerobic conditions (CSTR) (Fig. 13.7). The goal of this study was to see how substrate type affected biogas output. Different combinations of pre-treated dairy cow faeces and pear residue were co-digested (0, 25, 75, and 100%). In comparison to mono-digestion, the scientists found that adding a co-substrate increases biogas production significantly. In the combination (75% PLF + 25% LCM), a synergetic effect and good performance were seen, culminating in production of methane 390.2 ml/g and unstable solid clearance of

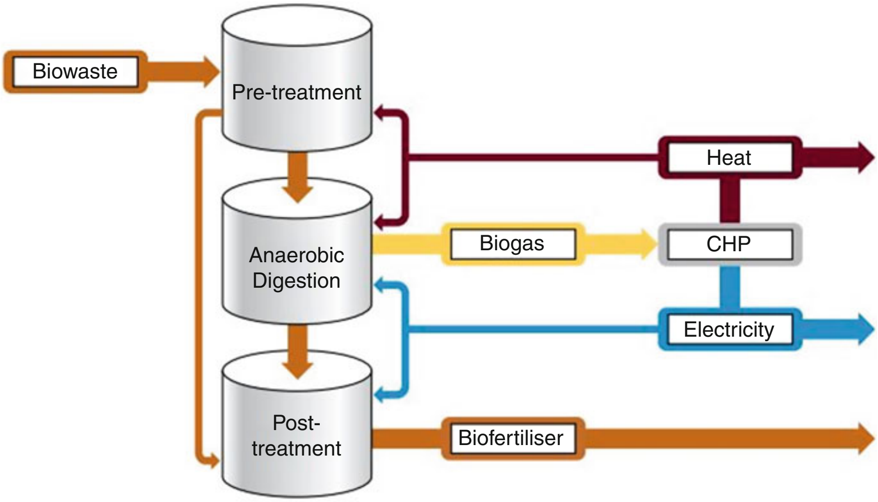

Fig. 13.7 Anaerobic digestion system

60%. The anaerobic digestion process was unaffected by the retention of pear waste. This study found that pear trash is a good candidate for co-digesting with cow dung and that the two may complement each other in terms of energy generation and waste management (Dias et al. 2014).

The value of cow dung may be shown in the balance of alkalinity, nitrogen content, and phenolic chemical and fatty acid dilution. Small family-sized biogas digesters were considered to offer significant promise in societies where appropriate feedstocks are easily available. A number of countries began programmes, with China and India leading the way. The results have been varied, especially early on. Between 1973 and 1978, China's efforts resulted in the building of seven million household-scale digesters. However, issues with quality control and management resulted in a high number of failures. Recent concerted efforts have concentrated on areas believed to be the most promising for the technology. Biogas service stations and service organisations have been formed. Around 1994, five million household plants were in good working order. India's experience has been on a lesser scale, but the statistics are nonetheless impressive—nearly 2.8 million home plants had been installed by the end of 1998. The Ministry of Non-conventional Energy Sources in India estimates that 12 million digesters might be built. Furthermore, access to cattle dung and water that must be combined with slurry has proven to be more difficult than predicted in subsistence agriculture. However, where farming is done with more actively managed animals and where manure supply is abundant—as in growing feedlot-based livestock—possibilities are better.

Dareioti et al. (2010) studied that in mesophilic conditions (35 °C), the oxygen independent co-digestion of olive mill effluent complete with cow dung. For 19 days of hydraulic retention time (HRT), acidogenesis and methanogenesis processes were monitored and regulated independently utilising two continuously stirred tank reactors (CSTRs). The value of cow dung may be shown in the balance of alkalinity, nitrogen content, and phenolic chemical and fatty acid dilution (Li et al. 2019).

Torrellas et al. (2018) devised several methods for determining gas reactions from anaerobic co-digestion of cow dung and commercial leftovers (normal measures, hypothetical estimation, and adopted guidelines of IPPC). Natural gas production from a natural source such as cow dung (CM) was evaluated in a small-scale utilising expeditious co-digested and prior treatment with impurified glycerine, which is a suitable option for balancing the nutrients in cow manure. When a combination of cow waste and impurified glycerine was pre-treated with ultrasound, the greatest biogas production (0.59 m^3 CH_4/kg VS added) start.

Impact of using coffee-mash in an anaerobic co-digestion operation with cow dung for natural gas generation under mesophilic conditions was investigated. The biogas generated from trials was examined using FTIR to see whether there were any dangerous chemicals present that might harm humans or animals. The combination can be inferred to be appropriate for the co-digestion process. The finding is further supported by existence of the microbial community during the process, as well as the optimal C/N ratio and presence of the microbial population balanced nutrient (Corro et al. 2013).

Dry fermentation technique has numerous benefits over wet anaerobic digestion in regard of small amount of energy consumption. Wet digestion requires mixing with water, liquid inputs, and agitation. From start-up to full-scale, biogas production and electric energy generation was assessed. First, a dry fermentation facility generated 3,431,900 m^3 of biogas and 6905.604 MWh of electricity. From a wet process, the outcomes are equivalent to the biogas with a 365-day period. The dry fermentation process may produce biogas at the same rate and use the same amount of electricity as the wet digestion process in absence of oxygen. Dry fermentation factors like hydraulic retention time (HRT), rate of initial loading, and digestate recirculation should be adjusted to get best results. Furthermore, this technique can avoid the difficulties associated with wet anaerobic digestion, and little research has been done in this area (Chiumenti et al. 2018).

In anaerobic digester of various organic wastes, cow dung was also utilised as a source of microbial community (maize cob, mixed vegetable waste, castor cake, etc.). Cow dung is a biodegradable organic waste that, due to high amounts of nutrients and pathogens, should not be disposed of in landfills. Cow dung can contain up to 10 pound per tonne of potash, 5 pound per tonne of phosphate, and 10 pound per tonne of nitrogen. In several nations, natural waste is dumped in the water resources, where it biodegrades, causing eutrophication and heavy metal pollution of water resources, as well as a slew of other environmental and health issues (Neshat et al. 2017).

13.4 Conclusion

The relevance of cow dung is very important in digestion in anaerobic condition as a technique of organic treatment of various natural wastes to generate biogases and bio-fertilisers. From various researchers' views, all of the study articles included in this present work stated the beneficial participation of cow dung and validated utility of natural waste. Biogas plants are springing up all over the world, and they can be a long-term answer for protecting our natural resources while also providing a renewable, clean, and affordable source of energy. Because of the various parameters that regulate the anaerobic digestion process, it is still difficult, especially when adding diverse organic wastes with varied compositions, characteristics, and qualities. Now is the moment to be conscious of the key environmental issues and to search out new technology, new ideas, and new tactics in order to achieve a balance in the environment, industry, and health.

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Chapter 14
Animal Dung for Better Soil Health Management

Rekha Yadav, A. P. Singh, Lanunola Tzudir, Debika Nongmaithem, Wanda Entalyrose Susngi, and Boduvelu Rhakho

Abstract The capacity of soil to function as a living system can be improved by the addition of organic matter and which can be improved by use of animal manure. The improvement in various properties of the soil (physical, chemical and biological) is achieved by the application of animal manure. The compaction of soil and its bulk density is also reduced by the use of manure. Application of manure also increases the aggregate stability of soil, water infiltration and retention. It also aids to reduce soil erosion. Manures can fulfil a significant amount of the crop's nutrient requirements for nitrogen, phosphorous, potassium and other micronutrients. Use of animal manure helps in increasing cation exchange capacity (CEC) and maintains the pH of soil in normal range. The biological activity in the soil is also improved by the use of animal manure which finally helps in better cycling and availability of nutrients to the crops. Soil fauna like bacteria, fungi and earthworms are also more in soil where manure is applied because it helps in increasing the activity as well as number of such organisms.

Keywords Animal dung · Soil health · Physical, chemical and biological properties

14.1 Introduction

Animal's dung can play a vital role in preserving energy and fertilizing the soil as well as able to make fuel through dung cake to restored charcoal, firewood. By doing so, it prevents deforestation and minimizes the use of various synthetic fertilizers like chemical fertilizer, pesticides, insecticides, etc. to the soil. Soil erosion, desertification and unsustainable agricultural practices have always been remarkable for the cause of declining in productivity on some land. The farmers utilized farm animals (bullocks, cows, pigs, buffaloes, poultry, etc.) dung and urine to make the soil fertile and healthy, enhance the productivity of the crop yield and reduce soil erosion and sustainable agricultural practices (Kesavan and Swaminathan 2008). Most of the

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_14

farmers in India are practicing mixed farming where the livestock are fed on local resources, for example, crop residues, pastures, shrubs, fodder trees and nutrients from forests and support lands are recycled back to cropland as manure. This type of manure helps to improve texture and decompose litter more easily (Kesavan and Swaminathan 2008). In ancient time, our country India were practicing as a traditional, using cow dung and urine as manure in crop cultivation (Ajayi 2007). However due to population growth, production of crops needs to be increased which resulted in increased use of commercial fertilizers which retard the soil microbial population and disturb the soil texture, porosity, water holding capacity, cation exchange capacity (CEC) and nutrient content. From this, we can say that manure remains a vital source of nutrients in agricultural production (Bargali 2004). Therefore, in order to increase the best potential of organic manure, we need to have information about the nutrient content in animal manure which in turn depends on the types and age of animals, feed sources, housing type, soil available nutrient, temperature and moisture content (Verma et al. 2017). Use of organic nutrient sources and soil amendment is quite interesting because these are carbon (C) source which obtained benefits; it impacts on soil properties, conditioning the soil, increasing the productive potential of soil and upgradation in soil quality, and it also helps in mitigating the problems of changing climate. But the interest of people in cultivating the organic crops and sustainable agriculture have also been the part that contribute to an increasing demand in organic soil amendment (Antonious 2016). The addition of organic matter to soil is an excellent way to increase soil organic matter. Soil organic matter contributes to overall soil health—the soil's ability and sustainability to act as a living ecosystem. Healthier soils improve crop yields and reduce soil loss from both wind and water erosion and protect water quality by reducing contaminated runoff. Organic manures help plants by faster uptake of nutrients from soil, increase availability of nutrients in soil, reduce soil pollution, minimize soil erosion and degradation and improve nutritional security and many problems related to crop production.

The overall improvement in various properties of soils (physical, chemical and biological) is the result of increased use of animal manure (Doran and Zeiss 2000).

14.2 Physical Properties of Soil

The physical stability of soil and good environment for various soil organisms as well as plants is supported by different physical properties of soil like water holding capacity, water infiltration, bulk density and soil aggregate stability. When animal manure is applied for long time, it also helps in improvement of organic matter of soil. Thus compaction as well as bulk density of soil is reduced by the use of manure, and improvement in stability of aggregates, infiltration and retention of water is also observed. It also aids to reduce soil erosion and crop stress. In different kinds of soils, decrease in the bulk density has been observed by long-term manure application. Finally, due to decrease in bulk density of soil, there is improvement in soil porosity as well as reduction in the compaction of soil. Organic manure application helps in the improvement of soil quality by modifying soil physical property. It is a primary factor in the development and modification of soil structure. Tremendous increase in water-stable large macroaggregates is found after application of manure (Six et al. 2000). Increase in aggregate stability provides a healthy environment for plant development as it helps to facilitate root growth, water infiltration and retention (Doran et al. 1996). Thus in wet years, there are less chances of runoff and less problem of erosion also, similarly, more resistant to drought in the dry period. These features therefore make the soil more resistant to compaction and reduce erosion effects improving its health.

14.3 Chemical Properties of Soil

Soil chemical properties (nutrient content, pH, soil organic matter and CEC) are very crucial for good work of soils to help in development and various functions of plant and soil organisms. Addition of animal manure in different soil fertility programmes improves the soil chemical properties depending on the content of amendment. The direct effect is the addition of nutrients. Manures can fulfil a significant amount of the crop's nutrient requirements for nitrogen, phosphorous, potassium and other micronutrients. Management of manure is to be done in such a way that when one nutrient is applied in appropriate amount, then other nutrients should not be applied in extra dose. The recommendations for manure are prepared depending on chemical content of the manure, so it is very necessary to do a complete analysis. To overcome the problem of excess salt, it is advisable to monitor the salt content of the manure by soil testing. Improvement in organic matter of soil is found by using manure having high carbon (cattle manure). Improvement in organic matter results in more availability of nutrients. Furthermore, improvement in organic matter of the soil can increase the soil CEC, and these also have potential liming effects on the soil (Morlat and Chaussod 2008). In this way, soil pH remains in normal range and also increases the nutrient availability to the plants (Eghball 2002).

14.4 Biological Properties of Soil

Soil biology consists of different living organisms within the soil (i.e. earthworms, fungi, bacteria, actinomycetes, algae, protozoa, nematodes, mites, springtails and small insects). A very crucial role is played by these organisms in making good health of the soil, in better decomposition of organic matter, better cycling of nutrients as well as aggregation of the soil particles. Due to such results, it is concluded that these living organisms help in increasing the nutrients' availability which can be easily absorbed by the plants for their better growth. Animal manure results in improvement of biological activity of the soil, favouring nutrient cycling and their abundance for the plants and finally producing “glue” essential for the stability of soil aggregates. More direct result on soil biological activity is observed by the use of animal manure when these are used at the same nutrient rate in comparison to inorganic fertilizers. In terms of organism’s abundance and activity, manure and bio-solid application increases the abundance of soil fauna such as bacteria, fungi and earthworms. The respiration rate and biomass carbon in microbes was found to be more by the use of manure, which are signs of abundance of microorganisms in the soil. From the results of some experiment, it is concluded that biomass of microbes is improved by two to three times due to carbon and some other nutrients present in the manure (Gomez et al. 2006). Grazing on microbes by micro arthropods and nematodes also release much of the nitrogen that is afterwards used by the crop in the growth period. Increased biological activity, i.e. microorganism activity, further helps in decomposition of organic matter.

14.5 Conclusion

Thus, overall soil health contributed by various physical, chemical, biological properties of the soil is all very important. These practices aid to enhance or maintain the quality of agricultural land. By using animal’s dung although it provides to crop as a slow-release fertilizer, it ensures healthy soil and also human health. As we know that commercial fertilizers deteriorate not only the soil properties but as well as human health. So, awareness and sensitiging the farmers that organic manures that comes from animals’ dung is very necessary to produce organic crops for sustainability of future generation (Verma et al. 2017; Magdoff and Van Es 2009).

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Chapter 15
Managing Cow Manure for Clean Energy: An Approach Towards Sustainable Conservation

Ayushi Sharma, Saurabh Gupta, Alok Bhardwaj, Anjana Goel, Kundan Kumar Chaubey, and Shoor Vir Singh

Abstract In India, cow dung is a low-cost and widely available bioresource used for a variety of traditional purposes, including fire, insect repellent and cleaning. Cow dung contains a varied array of bacteria that, due to their ability to produce a variety of metabolites, may be helpful to humans. Many cow dung microbes have proven inherent potential to boost soil fertility by phosphate solubilization, in addition to producing unique compounds. Nowadays, there is a growing interest in researching applications of cow dung microbes for biofuel generation and environmental pollution management. Anaerobic digestion is a process that converts organic wastes such as cattle manure, dairies solid wastes, etc. into methane and agricultural residues as a sustainable bioresource (biogas and biomethane). The composition of biogas produced by anaerobic digestion varies, but it primarily consists of methane and carbon dioxide, with a small amount of trace gases. The type of substrate being digested, operating temperature, pH, hydraulic retention duration, organic loading rate and digester design are all elements that influence biogas composition. As a result, the goal of this review study is to provide a complete examination of cattle organic waste (cow dung) that has been utilized as a substrate for the long-term production of biogas and/or biomethane, as well as its subsequent applications.

Keywords Cow manure · Biogas · Biomethane · Anaerobic digestion · Microorganism

15.1 Introduction

Humans' strong reliance on fossil fuels causes crude oil prices to rise from time to time, and they do so at a faster rate. For oil-importing countries like India, this is a big burden. Oil and gas remain India's primary energy sources. Oil reserves will

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_15

dwindle if we continue to consume fossil fuels. Renewable energy is required as a result of this. Biogas is one of the renewable energy sources. These gases are produced by anaerobic digestion of a variety of organic wastes, including biomass waste, human waste, and animal waste, and can be used as energy. In the absence of proper disposal procedures, livestock manure, like cow dung, can cause a variety of environmental and health issues, including pathogen contamination, odour, airborne ammonia, greenhouse gas emissions, and so on (Harikishan and Sung 2003). Anaerobic digestion is a waste-to-energy method that is frequently used to treat various organic wastes, such as the organic part of municipal solid waste, food waste, sewage sludge, animal manure, and so on (Li et al. 2009). Anaerobic treatment entails the decomposition of organic matter in the absence of free oxygen, resulting in the generation of methane, carbon dioxide, ammonia, and other gases, as well as low-molecular-weight organic acids (Lopes et al. 2004). Recently, the amount of cow dung produced by feedlot farming has increased dramatically, with the majority of it being disposed of in landfills or put to the ground without treatment. Anaerobic digestion is an alternative for waste treatment and energy recovery.

Bioresources like biogas and biomethane production from animal manure, particularly cow manure, has a lot of potential and benefits, including its environmentally friendly energy emersion. Additionally, the usage of livestock waste producing biogas slurry after processing produces rich organic fertilizer, essential for plant growth (Ginting 2007). Biogas can also help to reduce greenhouse gas and other pollution in the atmosphere. The present study illustrates the behavioural action of manure composition, rumen and water for during biogas production. The state of the digester, pH, nutrients, temperature, the C/N ratio and starter are all elements that influence biogas output (Khasristya 2004). The anaerobic digester must be kept in a state of equilibrium and active. The pH level should be kept between 6.6 and 7.6 since bacteria that produce methane can only function in this range (Metcalf 1979). Microorganisms require a temperature of 30 °C–38 °C for mesophilic and 49 °C–57 °C for thermophilic microorganisms to break down the substance. In the biogas production process, the optimal C/N ratio is 25–30. The starter is a critical component in the biogas production process. It is used to speed up the organic material reforming process. Activated sludge or rumen fluid content is common starter in biogas production (Widodo et al. 2006).

15.2 Characterization of Cow Manure

To properly dispose of and/or utilize cattle dung, it must be characterized in order to determine its value as a feedstock, including heavy metal content, C/N, GHG emissions and elemental composition (Fig. 15.1). The physical and chemical features of cattle dung have been detailed in a number of research papers, as given below.

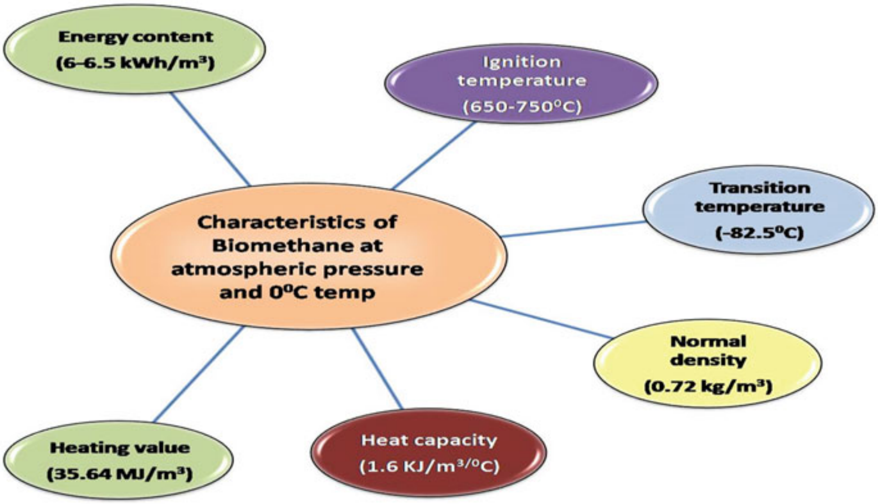

Fig. 15.1 Characteristics of biomethane produced from cattle organic waste

15.2.1 Moisture Content

The moisture content provided by different sources isn't always constant. Earlier studies opined variable percentages ranging from 13 to 75 wt%. Because energy is required to dry the material, the higher values will have a substantial impact on thermochemical conversion processes.

15.2.2 Ash Content

Due to the extreme high ash content of cattle dung (10.8–45.2 wt% dry weight) relative to wheat straw (4.7–10.3 wt% dry weight), ash-related difficulties such as agglomeration, sintering, deposition, corrosion and erosion may occur. A comprehensive evaluation of a wide range of temperature is suggested to analyse ash consequences during gasification of manure. The kind of bedding used refers to the amount of ash and its composition. Dairies in the United States employ fine and coarse sand, farm waste, sawdust and shredded papers, among other materials (USDA 2010). Sand is the widely utilized bedding materials as it promotes a clean and dry atmosphere, reduces growth of bacteria and provides pleasant place for cows to rest.

A stall base is utilized beneath the bedding to maintain cleanliness and minimize hock injuries. Concrete, rubber mats, mattresses, waterbeds and dirt are the most frequent stall base materials. When sand is used as bedding, the characteristics of dairy manure change dramatically, leading to a high ash content. Straw is commonly used as a sleeping material in the United Kingdom. According to the Farm Practices Survey guidelines (2016), an average mean of 1.41 and 0.32 tonnes per cow per winter for dairy cattle and slurry systems, respectively, should be employed (Smith and Williams 2016). The features of low and high ash biomass were described by Carlin et al. (2009). Diverse manure management strategies are responsible for the observed discrepancies. Cement-paved lots produce low ash manure. Moreover, a lot of the dung originates from earthen feed grounds, which have a lot of ashes in them. In flushing systems, recyclable solids screened mechanically have a lower ash proportion if sand was not employed as the bedding material. The sulphur concentration was found to be as high as 1.36 wt% (daf). During thermochemical process, sulphur in the organic compound can cause SOx emissions, which can cause acid rain.

Low melting temperatures are related with high alkali metal concentrations, which are unfavourable during thermochemical conversion. Despite discrepancies in manure management practices and an inadequate information on the bedding material employed, the ash compositions of two sources show identical amounts. The alkali index (AI) is previously utilized as a slagging and fouling indicator. Fouling and slagging issues may be expected for fuels with AI values greater than 0.34 (Shen et al. 2015). Ashes rich in macro- and micronutrients have the potential to be used as a soil fertilizer and amendment.

Certain metals are introduced to animal diets for health and wellbeing concerns; for instance, copper is provided to dairy cows' hooves to boost their condition, and a significant amount of the heavy metals absorbed ends up in excrement (Leclerc and Laurent 2017). Studies showed the heavy metal concentrations in solid and liquid slurry cattle manure. Heavy metals like lead (Pb), cadmium (Cd) and mercury (Hg) are of particular concern because they raise issue to health during human food chain. Plants grown on soils containing higher Cu concentration (upper limits 15 mg/kg dry matter) in bovine feed render them potentially unhealthy for animal and human consumption (Brugger and Windisch 2015). Mineral supplements supplied to feed are the reason for the difference in manure between dairy and beef cattle. Optimal Zn concentrations in beef cattle diets ranged from 22.0 to 777.0 mg/kg dry matter (DM), Cu concentrations from 3.7 to 61.5 mg/kg DM, Pb values from 1.0 to 4.99 mg/kg DM and Cd concentrations from 0.1 to 0.79 mg/kg DM (Nicholson et al. 1999). Earlier studies display the heavy metal concentration restrictions set by various governments in compost. Slurries have increased heavy metal contents, with Cu, Cd and Zn concentrations exceeding the compost limitations.

15.2.3 Volatiles

The chemical concentrations of cow dung on a dry and ash-free basis are constant with carbon concentrations of up to 50.0 wt percent dry basis. The high volatile matter content of cattle manure (50.2–64.6 wt percent dry basis) makes it suited for gasification.

15.2.4 Energy Content

Cow dung has a heating value of 13.5 MJ/kg (dry basis) that is just about half that of sub-bituminous coal or lower-grade coal (16–24 MJ/kg).

15.3 Role of Cow Manure as a Bioresource for Sustainable Development and Conservation

In India, cow dung is used in agriculture as manure, biofertilizer, biopesticide, pest repellent and energy source (Dhama et al. 2005) and works as a natural purifier for all wastes, according to ayurveda (Randhawa and Kullar 2011). As a result, in India, the cow (*Bos indicus*) is considered as "Gaumata" (mother of all) and "Kamdhenu" (a milch-producing species) (Jarald et al. 2008). Cow dung is generating a lot of curiosity throughout the world, and there have been a few attempts to harness its potential in the fields of energy generation and therapeutic items (Fig. 15.2).

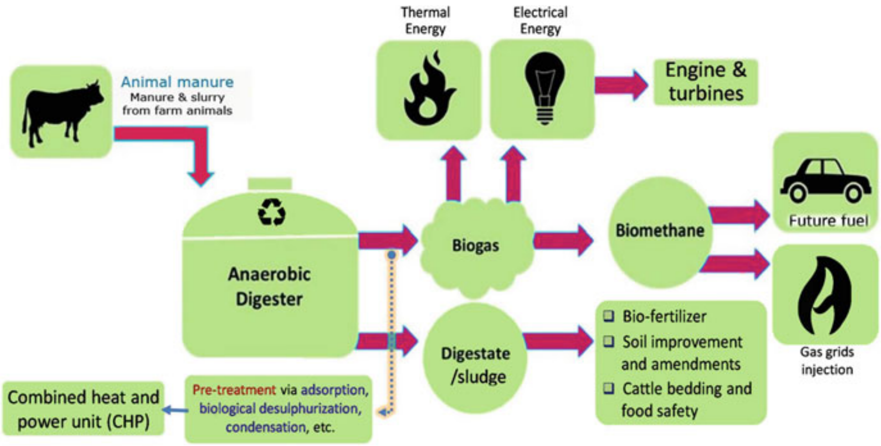

Fig. 15.2 Utilities of bioresources produced from cow manure for sustainable conservation

15.4 Source of Energy

The world's reliance on non-renewable energy sources such as coal, oil and gases is growing. Coal is India's primary energy source, accounting for 44% of total energy consumption. Despite being the world's third largest coal producer, our country is currently experiencing a coal shortage. Our reliance on imported fossil fuels has increased to 38%, according to the Energy Information Administration (EIA) (USEIA 2014). Due to the scarcity of coal, a readily available, cost-effective and environmentally acceptable renewable energy source is necessary. According to the Food and Agriculture Organization (FAO) of United Nations, animal manure produces 55–65% methane, which when released into the atmosphere can cause global warming at a rate 21 times faster than CO_2. Biogas is a combination of methane (50–65%) and CO_2 (25–45%) and other traces of gases produced by anaerobic digestion of organic materials by methanogens (Sharma 2011). With an equal amount of H_2O and a hydraulic retention time (HRT) at 24–26 °C for 55–60 days, one kilogram of cow manure can create as much as 35–40 litres of biogas (Kalia and Singh 2004), and anaerobic digestion of cow manure yielded 67 mL/gm methane, according to Li et al. (2009). In association with photosynthetic bacteria found in cow dung, bacteria such as *Azotobacter* and *Pseudomonas* species, other purple non-sulphur and sulphur bacteria are able to create the methane gas (Rana et al. 2014). Temperature ranges like 32–38 °C (mesophilic) and 50–56 °C (thermophilic) are essentially required for optimal methane generation (Kashyap et al. 2003). Because mesophilic bacteria cannot thrive in psychrophilic temperatures, biogas production in hilly places is reduced by 70.0% during the winter (Kanwar and Guleri 1994). This could be attributed to a drop in cell energy, the outflow of intracellular chemicals or mesophile cell lysis at lower temperatures (Gounot 1986). However, utilizing modest changes, numerous research observed a reasonable quantity of biogas generation in the psychrophilic temperature range (Safley and Westerman 1990; Kanwar and Guleri 1994).

In India, cow dung is the most common source of biogas ("gobar gas"), and there are a total of 192.49 million cow (indigenous or non-descript breed, 142.11 million, and Exotic or crossbreds, 50.42 million) (Livestock Census 2019). Cow dung produced by three to five cattle per day can power a basic 8–10 m^3 biogas plant that can produce 1.5–2 m^3 biogas per day, enough to feed a family of six to eight people, cook two or three meals, glow two lamps for 3 h and power a 2 kilowatt motor generator for 1 h (Werner et al. 1989).

15.5 Agriculture Management

Global population growth has resulted in intensive farming systems and inadequate cropland management, which has resulted in decreased soil fertility (Bedada et al. 2014; Onwudike 2010). Chemical fertilizers should be used extensively to restore nutritional shortages and boost crop output. Chemical fertilizers have a number of drawbacks, including increased soil acidity, its degradation and imbalance of mineral (Ayoola and Makinde 2008; Kang and Juo 1980), and even farmers no longer favour them (Bedada et al. 2014). Aerobic microorganisms breakdown complex organic matter into water, carbon dioxide, minerals and other stabilized organic matter during composting (Kala et al. 2009; Bernal et al. 2009; Vakili et al. 2015). Compost is used to boost the soil's nutrient content and water-holding ability (Vakili et al. 2015; Arslan et al. 2008). Researchers recently discovered that adding cow dung to palm oil industry biomass increases the physical and chemical qualities of compost, as well as the nutritional composition. The compost quality was greatly enhanced in terms of improved pH, C:N ratio and electrical conductivity by mixing the biomass of palm oil along with cow dung (in 1:3 ratio) (Vakili et al. 2015). Cow dung may thus serve as a soil conditioner as well as a substitute for artificial fertilizers (Garg and Kaushik 2005; Yadav et al. 2013; Belanger et al. 2014). Slurry from biogas plants is also a nutrient-dense source, but it cannot be used on a big scale due to issues like eutrophication and soil nutrient leaching (Wachendorf et al. 2005; Garg et al. 2005; Lu et al. 2012; Islam et al. 2010; Guo et al. 2014).

15.6 Bioremediation of Environment Pollutants

Through absorption, toxic substances entered the plant tissue, animals and humans (Adams et al. 2014). Active pharmaceutical ingredients (API), which are a mixture of several pharmaceuticals that are known to damage the water bodies or aquatic environment and agriculture runoff which includes animal waste, also contribute to the contamination of aquatic bodies that provide drinking water to humans (Kessler 2010). In India, only 10.0% of total wastewater is treated, with the rest being dumped untreated (Singh and Kohli 2012).

Cow dung contains a broad variety of microorganisms (*Acinetobacter*, *Alcaligenes*, *Bacillus*, *Pseudomonas*, *Serratia* species) making it ideal for microbial pollution degradation (Akinde and Obire 2008; Adebusoye et al. 2007; Umanu et al. 2013). Also, mixing slurry of cow dung in a 1:10 or 1:25 ratio was found suitable to degrade hospital wastes (in rural and urban areas), including oil spillage (Randhawa and Kullar 2011). Orji and his group found that bacterial and fungal species isolated from cow dung are critical for lowering total petroleum hydrocarbons (up to zero percent) in polluted mangrove soil (Orji et al. 2012). *Bacillus*, *Pseudomonas*, *Micrococcus*, *Citrobacte*r, *Flavobacterium*, *Vibrio* and *Corynebacterium* were among the bacterial isolates used in the process, while fungal isolates included *Aspergillus*, *Rhizopus*, *Fusarium*, *Penicillium*, *Mucor* and *Saccharomyces*. Adams et al. (2014) have explored the natural ability of above microorganisms in breakdown of hydrocarbons present in engine oil-contaminated soil. Metabolic activities of cow dung bacteria such as *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Flavobacterium*, *Enterobacter*, *Mucor*, *Trichoderma* and *Aspergillus* species reduced total petroleum hydrocarbon by up to 81%. According to Umanu et al. (2013), using cow dung in an acceptable concentration could be quite effective for biodegradation of water contaminants.

15.7 Human Health Management

Susceptible pathogenic bacteria can be killed or inhibited by microbial products or derivatives (Willey et al. 2008). However, pathogens have developed resistance to many antimicrobial drugs as a result of their overuse and misuse (Sharif et al. 2013; Aly et al. 2012). Antiseptic and disease-preventive qualities can be found in cow dung. It eliminates the bacteria that cause sickness and putrefaction. The use of five components, namely, ghee, milk, curd, urine and dung obtained from cows, collectively known as panchgavya supports various medicinal characteristics (Jarald et al. 2008; Pathak and Kumar 2003). Panchgavya therapy uses above five components alone or in combination with herbal drugs to treat a variety of bacterial and viral infections including flu, colds, cough, gastrointestinal tract disorders, skin infections, chickenpox, tuberculosis, leprosy, hepatitis and other illnesses like asthma, heart diseases, renal disorders, acidity, ulcers, wound healing and allergies (Jain et al. 2010; Dhama et al. 2013). Critical illnesses like cancer, diabetes and acquired immunodeficiency syndrome (AIDS) also seem to have benefit from panchgavya. Ayurveda also mentions pan-immunostimulatory, panchagavya's anti-inflammatory and immunomodulatory properties (Donovan 2008; Sathasivam et al. 2010; Girija et al. 2013). In albino rats, the effect of panchgavya on muscular tone, spontaneous motor activity and discomfort has recently been assessed (Paliwal et al. 2013).

15.8 Biogas and/or Biomethane Production Mechanism

Biogas (biomethane) is generated by anaerobic digestion. It is important to convert biodegradable waste into useful fuel, thus reducing the volume of waste products. Anaerobic digestion also assists in killing disease-causing pathogens. In anaerobic digestion, microorganisms digest organic materials in the absence of oxygen and airtight condition and at a certain level of moisture, temperature and pH (Angelidaki et al. 2003; Buren 1983). It is a multi-step biological process of acidogenesis, acetogenesis, hydrolysis and methanogenesis in which the organic carbon gets converted into CO_2 and methane (CH_4) (Fig. 15.3).

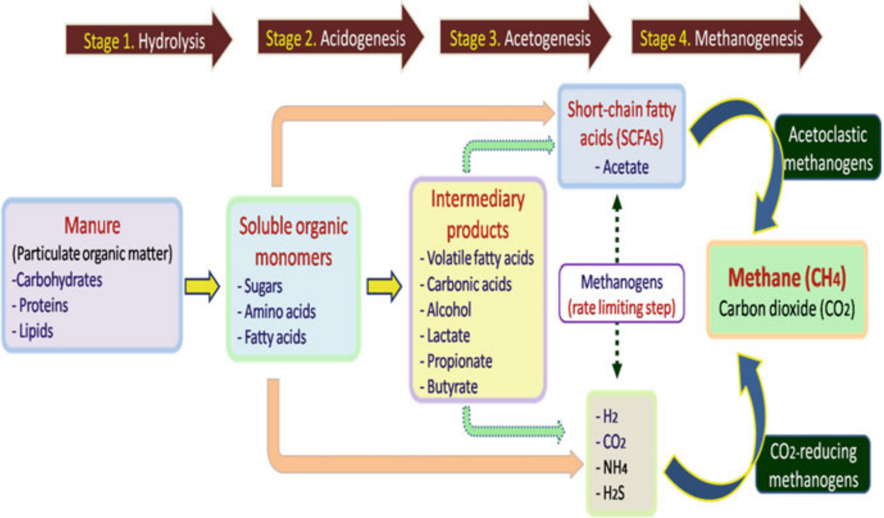

Fig. 15.3 Multi-stage biological process of methane production by anaerobic digestion

15.9 Hydrolysis

It is the primary or initial stage of biogas production through anaerobic assimilation. Facultative anaerobes (hydrolytic bacteria) break down complex organic compounds into soluble organic molecules, for example, proteins into amino acids, lipids to fatty acids and carbohydrates to sugars, by extracellular amylase, cellulase, lipase or protease enzymes (Parawira et al. 2005).

During anaerobic digestion, as reported by Vavilin et al. (1996), the presence of large particulate molecules with reduced surface to volume relation/ratio in the substrate may constrain the digestion reaction and make the hydrolysis itself a rate-limiting step.

15.10 Acidogenesis

The second step of anaerobic digestion is acidogenesis (Vavilin et al. 1996) in which facultative and obligate anaerobes or anaerobic oxidizers utilize the soluble organic molecules obtained from hydrolysis (Garcia-Heras 2003) and break them further into acetate, hydrogen and CO_2, which yields higher energy for microorganisms. According to Schink (1997) and Angelidaki et al. (2002), the products of acidogenesis consist of approximately 19% H_2/CO_2, 51% acetate and 30% intermediate reduced products, such as higher alcohols, lactate or volatile fatty acids (VFAs) which can be used directly by methanogens.

15.11 Acetogenesis

In acetogenesis, breakdown of short-chained, alcohols, aromatic fatty acids and higher VFAs to acetate and H_2 occurs. The products undergo the last step of the biogas production known as methanogenesis.

15.12 Methanogenesis

Methanogenesis is the terminal step of anaerobic digestion in which methanogenic archaea converts H_2/CO_2 and acetate to CO_2 and CH_4 (Kotsyurbenko 2005). Kotsyurbenko also reported the use of homoacetogenic bacteria in the CH_4 formation pathway, which is dependent upon the concentration of hydrogen in the system that oxidizes or synthesizes acetate.

15.13 Future Prospects

Biogas consists of 60–65% CH_4, 35–40% CO_2, 0.5–1.0% H_2S, some amount of water vapour and so on. It was found to be approximately 20% lighter compared to air. Biogas, like LPG, could not be converted to liquid form under usual pressure and temperature. Nevertheless, after removing CO_2, H_2S and moisture and then condensing it into cylinders, it was made easy for transport purpose and for immobile uses. On the basis of the availability of animal excreta from approximately 304 million animals, it was found to have an approximate capacity of 18,240 million m^3 of biogas production per annum. The growing number of pullet farms can be the alternative source as they could produce 2173 million m^3 of biogas per annum with 649 million birds. In addition, waste from kitchen, universities, institutes, restaurants, industries and so on in both urban areas and semi-urban areas and also nonedible de-oiled cakes from *Jatropha* provide immense capacity.

The biogas bottling project was planned to substitute manure and fuel worth 4000,000 per annum, in a span of 4–5 years. The extraction and bottling of CO_2 and separation of humic acid from the sludge would enhance the viability of Bio Gas Bottling Plants (BGBP). The BGBP gives three-in-one resolution of gaseous fuel production, organic fertilizer generation and wet biomass removal. The left sludge can be used as the organic fertilizer so as to improve the fertility of the soil.

Biogas is eco-friendly as it is devoid of foul odour, weed seed and pathogen. It contains many nutrients such as nitrogen, potassium and sodium (NPK) and micronutrients such as iron and zinc. The biogas plants prevent the emission of black carbon normally observed in households using *chulhas*. Biogas is a hygienic cooking fuel as it prevents CH_4 emission from unprocessed animal excreta and biomass ruins can be prevented. The biogas containing nutrients can be sealed in

CNG cylinders and used as and when required. These biogas plants are eco-friendly, and so these plants play the role as the most effective tool in checking the changes in climate.

15.14 Conclusion

Biogas has been used as the most appropriate machinery for several decades, allowing suitable usage of the available resource. It has been found to be a clean, hygienic and easy-to-use fuel at minimal cost, in addition to being completely environment friendly. Women living in villages no longer need to spend hours and travel long distances to gather firewood for cooking and burning purposes. They can now use this time for other activities. A smokeless and also a soot-free kitchen would mean that the women are not at risk of lung and throat infections and can live a longer, healthier life. Biogas has the capacity to meet all the fuel demands of houses, agricultural lands and industries. For example, biogas can be used for cooking, heating, lighting and so on. In farms, it can be utilized to dry crops, pump water during irrigation and so on. A noticeable advantage of this is that it saves firewood. With the setting up of biogas plants, employment chances are also created in villages. The utilization of biogas increases as the biogas plant generates fuel and fertilizers. The most important advantage of the biogas plants is that it could digest any wet mixture of waste, fertilizer and plant leftovers because of complicated bacterial methods. It does not cause reduction in the ammonia nitrogen content at the time of anaerobic digestion and helps in killing pathogens and weed seeds. In total, India has an immense capacity to produce electricity and heat from the waste as biogas. Till date, only a small part of the total capacity is used, and more investment in the field could increase the exploitation and help in realizing its true capacity in the near future.

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Chapter 16
Enzymes in the Digestion of Domesticated Animals

Anne Bhambri and Santosh Kumar Karn

Abstract Digestive enzyme is one of the important enzymes produced in the stomach and pancreas and released into the duodenum and helps to digest the food. There are several enzymes like proteolytic enzymes, gastric enzymes, pancreatic enzymes, glycolytic enzymes, salivary and pancreatic amylase enzymes that are discussed in this chapter. In the digestive tract of cattle, the functionality and composition of the microbiome or microbiota are considered robust. The high grain feeding decreases the diversity, richness and functionality of this microbiota and hence affects the production and health of animals. Gut microbiota contributes to the metabolism of host as it protects against the pathogens, educates the immune system and, via these basic functions, directly or indirectly affects most of the physiological functions of its host.

Keywords Digestive enzymes · Microbial diversity · Gut microbiota · Microbiome · Gastrointestinal tract

16.1 Introduction

The production of digestive enzymes is primarily done in the stomach and pancreas and then released into the duodenum, i.e. the first part of small intestine to help in the digestion of food coming from the stomach. Protease, lipase and amylase are the main types of enzymes that helps in the breakdown of macronutrients in food, i.e. helps in the breakdown of proteins, digest fat and process carbohydrates correspondingly. There are most of the mammals that produce amylase in their saliva, but dogs and cats do not produce. This reflects their expected meat diet and organs from prey.

In case of herbivores and omnivores, they have flat molars which crush and chew the food, but carnivore's dentition is designed perfectly to capture and kill the prey

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_16

and to rip and tear the meat from the bones. However, carnivores do not spend much of the time in chewing, nor they consume many of the carbohydrates, that's why they do not need for amylase in the mouth. Moreover, cells also carry lysozyme enzymes that released upon cell death and help in the digestion or breakdown of the contents of the cells, either by decomposition or for recycling in a living body as the natural prey diet of canines and felines consumed the raw and these lysozymes also contribute to efficiently digest the food.

When carnivores kill large prey animals, then carcass is likely to sit for a period, i.e. it takes around 2 weeks for mountain lion to consume the whole deer. Moreover, commonly, wild canids take the advantage of lysosomal process via burying the parts of carcass and further digging them up and then eating them weeks later. In those plots, both the internal lysozymes and several external organisms, i.e. fungi, bacteria, etc., contribute to the breakdown of food that is normally performed by the pancreas. They become non-functional when heat destroys the natural shape of the enzymes. Those enzymes are not present in cats and dogs which eat heat-processed pet foods; therefore, the pancreas must provide all the enzymes needed in the digestion of food.

Moreover, carbohydrates are much higher in commercial dry kibble than the natural prey diet of carnivores, whereas amylases can or do produce in the intestinal and pancreatic tissues of cats and dogs which are fully capable to digest the carbohydrates. The nature did not intend the carbs to be their primary source of nutrition due to lack of salivary amylase. Digestive enzyme's production is independent of diet shown in much research in animals. In response to food ingestion, animals are biologically programmed to produce specific amounts or types of digestive enzymes regardless of what type of food they eat. However, only major evolutionary shifts like changing from omnivores to insectivorous lifestyles affect these systems. Carnivorous pets cannot adapt their digestive functions to processed diets that after all have only been used widely for few decades (Sissons 1981). The emerging technology which shows the potential in terms to improve the utilization of forages by ruminants is the use of exogenous cell wall degrading enzymes. Research shows that to improve the cell wall digestion, supplementing dairy cow and feedlot cattle diets with fibrolytic enzymes has significant potential (Nakashima et al. 1988; Feng et al. 1996; Hristov et al. 1996; Yang et al. 1999; Colombatto 2000).

Elevated evidences show that by adding exogenous fibrolytic enzymes to ruminants, diets improve the production of milk of dairy cows and gain weight of beef cattle but the results are variable particularly when viewed across all the products (Beauchemin et al. 2001). After it is viewed, it demonstrated that feed enzyme diet supplementation is not so suitable technique for the ruminants. The variability may be attributed to some factors like inappropriate technique to provide the product of enzymes to the animals, product formulation, diet composition, target animal's energy status, under or over-supplementation of enzyme activity and many other factors.

Most of the commercially available product of enzymes are evaluated as ruminant feed additives that are produced for non-feed applications (Bhat and Hazlewood 2001). Limited amount of ruminant enzyme products available commercially in terms of advancing the enzyme technology, much progress has been made for ruminants, and to develop the more effective products of enzymes, considerable research is still required.

For ruminants, a feed enzyme contains mainly the activity of cellulase and hemicellulose and is of fungus (especially *Aspergillus niger*, *A. oryzae* and *Trichoderma longibrachiatum*) and bacterial like *Bacillus* sp. origin (Pendleton 2000).

16.2 Enzymes Involved in Digestion

16.2.1 Proteolytic Enzymes

In the gastric lumen, the enzymatic hydrolysis of dietary proteins occurs under the action of HCL and Pepsins (Seijfeers et al. 1963). Further, in the intestine, hydrolysis gets continued where peptides and proteins from gastric hydrolysis are submitted to the action of intestinal peptidases and pancreatic proteolytic enzymes (Gray and Cooper 1971; Heizer et al. 1972).

16.2.2 Glycolytic Enzymes

The hydrolysis of dietary carbohydrates is done by salivary, mainly pancreatic amylases. They get hydrolysed in the intestinal mucosa by disaccharides. In germ-free animals, there is no cellulase in their intestine and this enzyme being of microbial origin.

16.2.3 Pancreatic Amylase

In germ-free animals, chickens and conventional rats, there is no difference in the level of pancreatic tissue amylase activity (Lepkovsky et al. 1966; Reddy et al. 1969; Coates et al. 1970). In the distal part of the conventional animals like rat, chicken and rabbit, the pancreatic amylase is partly inactivated (Lepkovsky et al. 1964; Yoshida et al. 1968). On the contrary, in the distal part of the conventional rat's intestine, Lepkovsky et al. (1966) found higher activity of enzyme, while according to Borgstrom et al. (1959), there is no difference showed between the faecal amylase activities of germ-free and conventional rats.

16.2.4 Gastric Enzyme

In the stomach, the hydrolysis of proteolytic enzyme is performed mainly by the pepsins that secreted in an inactive form called pepsinogens and activated by the HCL, but there is no data available on secretion and pepsinogen biosynthesis in the germ-free animals. In germ-free animals, the basic secretion of HCL was higher (X 2.7) than that of the conventional rats, but the basal gastrinaemia in these animals was not different (Roze et al. 1977).

16.2.5 Pancreatic Enzyme

Trypsinogen, chymotrypsinogen, proelastase and procarboxypeptidases A and B are the pancreatic proteolytic enzymes that are secreted in an inactive form by the exocrine pancreatic cells and by an intestinal enzyme, i.e. enterokinase, which gets activated in the intestinal lumen. In both the germ-free and conventional states in the rats and chickens, the tissue levels of trypsinogen and chymotrypsinogen appear to be similar (Lepkovsky et al. 1966; Reddy et al. 1969; Coates et al. 1970).

16.3 Role of Microbes in Digestion or Microbial Diversity of Gut

The digestive role of different microbes is different. In the rumen, there are three different types of microbes produced, i.e. bacteria, protozoa and fungi.

In the rumen, the elevated production of microbes is the key to life of the production of milk and composition. These microbes produce volatile fatty acids by the breakdown of feed that are used by the cow as energy for milk production and maintenance. In the dairy cow, rumen microbes are absorbed and digested in the small intestine as the main protein source for the production of milk that provides up to 70–90% of cow’s protein.

Rumen microbes have two main groups, i.e. the fast-working microbes and the slow-working fibre. The fast-working microbes float around in the fluid of rumen, looking for easily digested nutrients such as starches and sugars, whereas the digesters of slow-working fibre are located on the fibre mat in the rumen. Each group of microbes have their specific functions such as starch, protein, digesting fibre and sugar, and the amount of each of them is directly related to the diet. The types of feed mainly the content of fibre influence the digestion speed, microbe type in the rumen and the total intake of nutrients and dry matter.

The population of microbes takes time to build up and recover after the sudden changes in feed such as for forage fibre, the digesting microbes take about 4–6 weeks to build up where as for starch; the digestive microbes take around 4–5 days to build up; and in lactic acid, the bacteria produced from slug feeding take 2–4 h to build up.

There are some important nutrients that are required for the microbes: these are water that maintains the liquid environment in rumen and supports the metabolism of microbes and also dilutes the acids in the rumen; minerals (sulphur, magnesium, phosphorus and calcium) that are important in the growth and multiplication of microbes; and energy (starch like cereal grains; digestible fibre like palm kernel extract, forages, cotton seed hulls and brewer's grain) required for the growth and multiplication (Queensland Government 2021).

Worldwide, the potential of dairy cow's milk production has elevated substantially in the recent years. To meet the potential of these cows, they require high energy. This is achieved often by feeding more concentrates like grains and less forages. Nevertheless, these diets may adversely affect the microbiota of the digestive tract which affects both functionality and composition of the microbiota and lead potentially to the colonization of the opportunistic pathogens (Russell and Rychlik 2001; Plaizier et al. 2008; Krause et al. 2013). On microbial symbiosis, cows rely with the microbiota in their intestines and rumens as due to these microbiota, cows able to digest fibre, they convert non-protein nitrogen into protein, synthesize vitamins and also break down the toxic compounds in digest a (Russell and Rychlik 2001; Krause et al. 2013). Consequently, the adverse conditions for gut microbiota affect the production, health and welfare of cows.

16.4 Microbiota in the Digestive Tract of Ruminant

Immunologically active organ system of ruminant is the digestive tract that is constantly exposed to a multitude of exogenous and endogenous stimuli. To the diverse and complex ecosystem, gastrointestinal tract is the home of microbes called as microbiome or microbiota. On earth, the one of the most complex microbial ecosystem is the gastrointestinal tract of animals that is continuously affected by the factors associated with the host (Spor et al. 2011) and outside environment (Claesson et al. 2012). The amount of microbial species present in the gastrointestinal tract of ruminants can be varied depending upon the feeding strategy, geographical location and diet, and it has been estimated to be more than 5000 (Henderson et al. 2015).

Gut microbiota are composed of viruses, eukaryotic organisms, bacteria and archaea that reside in the gastrointestinal tract. In a symbiotic fashion, it relates with the host like in the gut; bacteria produce short-chain fatty acids which nourishes the epithelium of intestinal, whereas epithelium produces the mucus which feeds beneficial bacteria. Gut microbiome or microbiota has some metabolic functions including it protects against the pathogens and educates the immune system, as well as via these basic functions, it also affects directly or indirectly to most of our physiologic functions. Serotonin is a neurotransmitter that is mostly produced in the intestine that has led to develop the concept of gut brain axis (O' Mahony et al. 2015). Microbiome which is stable and healthy may simultaneously act as pro- as well as anti-inflammatory and to prevent the excessive inflammation it keep balances and still able to respond to the infection (Tizard and Jones 2018).

Various ecological measures like abundance, diversity, richness and evenness are used to compare as well as describe the microbiotas among the animals and across treatment (Caporaso et al. 2010; Gotelli and Colwell 2010).

In the digestive tract, the gut microorganisms are different in their ability and functionality to use the fractions of the substrate resources (Levine and D'antonio 1999; Henderson et al. 2015). Consequently, high microbiota evenness, diversity and richness are considered to be as beneficial as it enhances the microbiota's stability, especially during the nutritional challenge condition, and allows it more efficiently to use the limiting resources (Russell and Rychlik 2001). A number of metabolic disorders in ruminants like acute and subacute ruminal acidosis are associated with the reduction in the diversity of hindgut and rumen bacteria (Khafipour et al. 2009; Azad et al. 2015; Plaizier et al. 2017) The richness of microbiota is elevated under some intestinal and extra-intestinal infections and inflammation like helminthic parasitosis (Lee et al. 2014) and bacterial vaginosis (Ling et al. 2010). In the digestive tract, the digesta contain pathogenic and commensal microorganisms and food residues that impose continuous immunologic challenge to the host animal (Russell and Rychlik 2001).The difference in the diversity and composition of normal microbiota that exist between the small intestine and stomach (abomasum) and between the hindgut (Colon and Cecum) and foregut (reticulum, omasum and rumen).

Lower number of few species of microbes resides in the upper small intestine (duodenum and jejunum) and stomach due to the specific features of digesta in the hindgut and foregut like propulsive motion of the region and acidity (Russell and Rychlik 2001; Penner et al. 2014). In intestinal and extra-intestinal diseases, the role of gut microbiome is emerging (Russell and Rychlik 2001; Krause et al. 2013).

The ruminant gut microbiota in other mammals is dominated by the microbial phyla, i.e. *Bacteroidetes*, *Proteobacteria* (mainly in the class *Gammaproteobacteria*), *Firmicutes*, *Verrcomicrobio* and *Actinobacteria* (Henderson et al. 2015) whereas most of the archaea as methanogens in the phylum *Euryarchaeota* (Hobson and Stewart 1997; Hook et al. 2010; Janssen and Kirs 2008; Singh et al. 2011). The different sections of the ruminant gut present differ in environmental conditions, and the composition of gut microbiota changes from one section to other. The first region is the foregut that houses various common fibrolytic species (Devillard et al. 2004; Morrison and Miron 2000; Suen et al. 2011) and also lactolytic and saccharolytic, proteolytic and amolytic species (Chistoserdova et al. 2004; Forano et al. 2008; Jiao et al. 2014; Marx et al. 2011). The second region is the abomasum that has pH of about 2–4 that digests and kills many of the microbes entering from the omasum and also supplying the host with 60–90% of its amino acids that in turn is absorbed in the small intestine (Asplund 1994; Stevens and Hume 1995, 1998). In the third region, for neutralizing acid from the stomach, the small intestine is responsible that breaks down the macromolecules with enzymes and also absorbing the nutrients (Frey et al. 2010). The last site is the hindgut for water and salt balance (Van 1994; Guo et al. 2008).

16.5 Conclusion

Current chapter summarizes and discusses the various enzymes such as glycolytic, gastric, pancreatic enzyme, etc. involved the digestion of animals simultaneously also discussed the gut microbial diversity and their role in the digestion process. The composition of microbial community is different among the regions of gut than between the dietary groups such as the physiological conditions with the digestive tract playing an important role in structuring the microbial communities than does diet.

Acknowledgments Authors are thankful to Dr. Gaurav Deep Singh, Chancellor, Sardar Bhagwan Singh University, Balawala, Dehradun, Uttarakhand, India, for providing facility, space and resources to conduct this work.

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Chapter 17

Roles of Beneficial Microorganisms for the Effective Production of Commercial Animal Feed

Charles Oluwaseun Adetunji, Olugbemi T. Olaniyan, Rashmi Dash, and Ajit Varma

Abstract The whole globe's population has been forecasted to increase drastically to 9 billion by the year 2050. Hence, there is a need to search for a sustainable solution that will meet the demand of the need to need the need of the ever-increasing population. Food insecurity and nutritional imbalance as well as the issue of climate changes are major challenges facing mankind. Also, the livestock industry is currently facing diverse challenges most especially the higher cost of animal feed which has led to the request for a sustainable substitute animal feed that could enhance a safe and nutritionally enhanced animal feed. The application of biotechnology through the application of beneficial microorganism most especially those derived from the rumen area of ruminant and non-ruminant animals has been identified as a sustainable solution that could help in production of a more nutritional enhanced animal feed which has a lower cost of production. Therefore, this chapter intends to provide comprehensive information on the application of beneficial microorganisms as well as the role of beneficial enzymes derived from these microorganisms for the production of animal feeds.

Keywords Microorganism · Biotechnology · Enzymes · Rumen · Animal feeds

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_17

17.1 Introduction

It has been reported that the total meat production has tripled from 45 to 134 million tons from the year 1980 to 2000 most especially in the developing counties (Thornton 2010). The demand for meat will increase only with 0.6% in developed nation in comparison with the yearly rise of 2.8% in most developing countries (John and Maria 2001).

Therefore, there is a need to search for a sustainable solution that could entail the production of a more nutritious animal feed as well as to meet the demand of the ever-increasing population along with increases in wealth levels and the changes in dietary patterns toward primarily meat-based diet. The application of biotechnological techniques has been identified as a novel technology that could be applied for the effective production of animal feed (Bimrew 2014).

The application of biotechnology in animal feeds has been documented to enhance animal nutrition through the application of beneficial microorganisms. Also, the application of genetic manipulation could also enhance the utilization of rumen microorganisms so as to enhance animal health (Yadav and Chaudhary 2010; Shelke et al. 2011).

The application of biotechnology could also help in the production of animal feed through adequate conversion of numerous agricultural and environmental wastes into nutritional enhanced animal feeds (Adetunji and Anani 2021a, b; Okeke et al. 2021; Adetunji et al. 2021a, b, c, d; Adetunji et al. 2020; Olaniyan and Adetunji 2021). This will go a long way to improve the digestibility of the animal feed through the action of some crucial enzymes and decrease wastage of feed (Bimrew 2013). Also, the introduction of biotechnology has enhanced the genetic selection for feed efficiency as well as decrease the non-productive days for dairy (Capper 2011; Meyer et al. 2006; Klusmeyer et al. 2009; Connor 2015).

Hence, this chapter provides a comprehensive information on the application of biotechnology for the improvement of livestock feed production.

17.2 Direct-Fed Microbials (DFM)

The ingestion of yogurts enhances the level of productivity in animal because of the health-promoting probiotic effects (Adolfsson et al. 2004; Meydani and Ha 2000). Some other benefits entail prevention of animal feeds from spoilage action of microorganisms and play a crucial role in the nutritional enhancement of animal feed (Kung Jr et al. 1993; Sheperd et al. 1995). They also play a crucial role as a substitute to synthetic antibiotics where the beneficial microorganisms could improve the productivity of animal rearing (Danisco 2008).

The majority of such microorganisms are added as an ingredient to animal feeds, and they are sometime refer to as biologically derived extracts, which entails seaweeds, yeasts, enzymes, and essential oils. Numerous examples of incorporated microorganisms include *Aspergillus niger*, *Saccharomyces cerevisiae*, *Aspergillus* *oryzae*, *Enterococcus thermophiles*, *Bacillus coagulans*, *Enterococcus lactis*, *Bacillus coagulans*, *Enterococcus faecium*, *Bacillus licheniformis*, *Enterococcus diacetylactis*, *Bacillus pumilus*, *Enterococcus cremoris*, *Bifidobacterium adolescentis*, *Bifidobacterium longum*, *Lactobacillus cellobiosus, Propionibacterium shermanii*, *Lactobacillus bulgaricus*, *Pediococcus acidilacticii*, *Lactobacillus curvatus*, *Lactobacillus casei*, *Lactobacillus plantarum*, and *Pediococcus pentosaceus* (Fuller 1989).

17.3 Mechanism of Action of DFM

It has been observed that some of these beneficial microorganisms could relate directly with the immune system and the host epithelia system, while some other microorganisms portend the capacity to regulate their activity by regulating the intestinal flora available and play a crucial role in mitigation of pathogenic microorganism. The mode of action through which these microorganisms perform their role can be categorized into three major groups which entails microbially mediated immune-development, competitive exclusion, and chemical inhibition.

It has been established that intestinal bacteria possess a significant influence on the immune-development of the gastrointestinal system and portend the capacity to provide antigenic materials that could enhance the growth of Peyer's patches (Klaasen et al. 1993; Rhee et al. 2004; Rothkotter and Pabst 1989; Štěpánková et al. 1980), gut-associated lymphoid tissue (Gaskins et al. 1996; Snel et al. 1998), generation of protective IgA molecules (Langford et al. 2002; Macpherson and Uhr 2004), and the production of antimicrobial peptides (Ayabe et al. 2000; Ismail and Hooper 2005).

17.4 Significance of Biotechnology in Feed Enhancement

17.4.1 Refining Nutritive Value of Cereals

It has been validated that low amounts of specific amino acids and moderate protein content could affect the nutritive contents of cereal by-products and cereals (Scott et al. 2012). This has been recognized as a significant demerit most especially in the ration formulation for most non-ruminant livestock that warrants the incorporation of exclusive protein supplements. The low level of lysine in barley most especially through the action of genetically engineering the grain genome could be improved through diverse biotechnological techniques (Scott et al. 2012).

17.4.2 Eliminating Anti-nutritive Factors from Present in Feeds

There are numerous antinutritional factors that are available in plants such as sanapine, glucosinolates, and tannins in oilseed rape, protease inhibitors, and cyanogens in legumes. It has been discovered that the presence of amino acid deficiencies and the detrimental influence of these compound in animal feeds could become more conspicuous in non-ruminant animal when compared to ruminant animal (Adeyemo 2013).

17.5 Metabolic Modifiers

Metabolic modifiers such as recombinant bovine somatotropin have been applied to improve the level of composition enhancement and weight gain in animal. Also, this has also resulted in productive enhancement such as increase in weight gain, higher level of milk production, and reduction in the animal waste per production (Roland 2013).

17.6 Improving the Process of Digestibility of Low-Quality Forages

It has been documented that that action of microorganism could play a crucial role in the broken down of low-quality forages most especially in the rumen region into nutritionally enhanced feed. The process could also improve the level of digestibility of the animal feed. The action could minimize the outflow rate of the feed residue that is derived from the rumen compartment which subsequently decreases feed consumption of the animal (Lazzarini et al. 2009). The conventional techniques applied in the treatment of low-quality forages like cereal straws involve a range of chemical treatments, grinding, pressure, and temperature treatment. The process involved in lignification of the cell walls could preclude degradation feed through the action of cellulase enzymes.

17.7 Increasing the Role of Rumen

It has been observed that transgenic technology could play a lot of function on the activities of the rumen microorganism. This could be linked to the activities of transgenic bacteria that portend the capability to improve cellulose broken microorganism that could break down hemicellulose complexes more easily, improve the potential of these microorganism for specific amino acids generation, and enhance potentials for nitrogen contents and higher potential to decrease methane generation. Moreover, the introduction of genetically engineered rumen bacteria could help in all these processes (Malmuthuge and Guan 2017).

17.8 Beneficial Microorganisms that Are Used in Commercial Production of Animal Feeds

Bernardeau and Vernoux (2013) reported that several microbial adjuvants like probiotics, beneficial microbes, and other zootechnical additives can be utilized to supplement human and animal diets. The authors reported that many beneficial microorganisms are added to feed to improve animal performance and productivity, reduce toxicity, and enhance nutritive value.

Komar and Yaser (2019) utilized different feed additives to achieve sustainable growth performance and livestock production. In their study, it was discovered that the feed additives are good source of anti-infection agents which are very safe and healthy. The authors showed that the feed additives are of different types such as (1) acidifiers, natural and salt of acids or inorganic acids like formic acid, lactic acid, citric acids, fumaric acid, hydrochloric acid, phosphoric acid, sulfuric acid, calcium-formate, sodium diformate, sodium-fumarate, and potassium-diformat; (2) minerals like macro-minerals, trace elements (Fe, Mn, Zn, Cu, Se, I, Co, Mo, Cr, Ni), and micro-minerals; (3) probiotics such as non-bacterial and bacterial probiotics, multi-species and single-species probiotics, non-spore forming and spore forming bacteria, and autochthonous and allochthonous probiotics; (4) prebiotics like inulin, transgalacto-oligosaccharides, fructo-oligosaccharides, and lactulose; (5) synbiotics like both prebiotics and probiotics; (6) ionophores such as monensin and propionate; (7) nucleotides and (8) phytogenic feed additives like plant-derived extricates, e.g., essential oils, carvacrol, capsicum oleoresin, cinnamaldehyde, and secondary metabolites, e.g., flavonoids, saponins, and tannins; (9) enzymes as feed additives such as cellulases, β-glucanases, xylanases, pectinases, proteases, amylases, and phytases; (10) electron receptors like sulfate and nitrate; and (11) bacteriophages like disinfectants and antibiotics. The authors demonstrated that many of these feed additives are utilized as immune-stimulatory, hepato-protective, anti-inflammatory, antibacterial, antifungal, antiviral, and anti-parasitic purposes.

Imran et al. (2016) reported that digestive enzymes were utilized for feed supplements to enhance the nutritive value and reduce the anti-nutritive components present in feeds. They revealed that enzymes can increase the consistency and maintain the gut physiology of animals. Some of the enzymes highlighted were phytases, beta-glucanase, proteases, cellulases, and alpha-amylases. Many of the enzymes are utilized in the industry through microbial biotechnology by incorporation into the feed or used in the production of syrup. These exogenous enzymes have been reported to be widely used in poultry diets as supplementation and enhancing the nutritive value. Most of the poultry dietary ingredients include enzymes (amylases, p-glucanases, pectinases, arabinoxylanases, hemicellulases, cellulases, acid proteases, phytases, alkaline proteases esterases, and lipases), chemicals, and molecules like phytate, non-starch poly saccharides, phytin, and tannins.

Castillo-Gonzáleza et al. (2014) reported that in order to enhance the ruminant system, the use of microorganisms and other additives like monensin, buffers, tallow, nitrogen compounds, probiotics, and prebiotics to facilitate nutritional strategies through the manipulation of ruminal fermentation has become necessary. These processes allow the change in ruminant fermentation process for enhanced digestion, growth, and efficiency.

Ezema (2013) revealed that probiotics as a microbial-based constituent can be utilized for advancing diverse health benefits in animals. The author showed that many probiotics are nontoxic, non-pathogenic, and very useful for the digestive system, promote growth and development, and enhance the reproductive performance and metabolic functions of many host animals. Studies have revealed that the nutritional requirements for animals can be protein, energy, vitamins, and minerals which are incorporated into feeds.

Ravinder et al. (2015) reported that recently there is serious opportunity to search for alternative livestock nutritionists as feed additives through beneficial microorganisms. The normal intestinal microbiota in animals and the strains of probiotic fungal, bacteria, and yeast can be isolated and added to the diets. These microbes can develop and grow the rumen, stabilize the pH and production of end-products, enhance ruminant fermentation, stimulate digestion and nutrient flow, suppress oxidative stress, facilitate the immune response, eliminate pathogens, suppress acidosis, and increase animal products and vitamin synthesis. The digestive system of livestock fed with manufactured microbial feeds combined with microbes is indicated to enhance the natural flora and activity of the animals.

Salem (2010) reported that animals particularly found in arid and semiarid regions have severe nutritional deficits due to scarcity of food, diseases, and other health challenges. Thus, the author showed that ruminant-based improvement in production through innovative technologies in feed resources, naturally manipulated rumen with natural compounds enhancing microbial activity, and increased nutritional quality will ultimately increase the performance and productivity.

Afreen and Ucak (2020) reported that fish waste containing several species of microorganisms can be utilized for aquaculture and animal feed. The authors reported that environmental pollution because of fish waste has resulted in global concern; thus in order to overcome this challenge, they can be converted into animal feed due to their richness in vitamins, minerals, and protein. The by-products of the fish waste can be converted through biotechnology using fermentation method or techniques into animal feed.

Ritala et al. (2017) reported that as a result of the increase in global population, global demand for meat, food, and dairy products have increased tremendously necessitating the urgent revitalization of livestock and dairy production to feed the ever-increasing population. The authors noted that single-cell protein derived from algae and microbes can serve as alternative sources of additives and animal feeds to improve the protein contents. Several authors have revealed that microbes can be utilized in food and feed industry to enhance the nutritive value of animals and humans through secretion of metabolic compounds, biological inhibitors, and various preservative constituents. These constituents and microbes are very rich in protein which can be easily cultivated, have very low gestation, and are highly nutritive. Recently, studies have shown that chemical stimulators or stimulants are toxic and not safe as additives in feed industries. Naturally occurring microbes can serve as alternative sources of feed additives which can improve productivity, digestion, prevention of acidosis, weight gain, quality of products, stability of pH, immunomodulation, and health of livestock (Adedayo et al. 2011).

Livestock production, which represents the largest and fastest-growing sector in agriculture, has been reported to contribute significantly to food industry. Thus, various approaches have been suggested to help in enhancing the capacities of this sector such as the utilization of microbes as probiotics in improving livestock productivity, growth, nutritive value, and performance. Probiotics containing microbes as feed additives are known to enhance livestock gut physiology, growth, prevention of enteric infections, and increased nutrition.

ASML et al. (2015) reported that in the past 15 years, there has been significant increase in the utilization of probiotics in animal feeds. These probiotics are made up of microorganisms with significant health benefits through the elimination of pathogenic organisms and growth, serious competitive advantage in the host for nutrients, release of antimicrobial constituents, deactivation of toxin, stimulation of immune response against infections, and active adhesiveness. Linder (2019) reported microorganisms are good sources of vitamins, single-cell protein, single-cell oils, mineral, biomolecules, and protein which have huge physiological benefits to the animals when incorporated in their feed.

Sari et al. (2019) reported the utilization of different types of animal feeding feed additives to increase performance effectiveness in animals. The authors revealed that high-fiber diets of various composition were administered to the animals, and from their results, it was revealed that the probiotics changed the rumen microbial metabolic characteristics, enhanced growth, and reduced pathogenic organisms and formation of hydrogen peroxide and lactic acids (Yirga 2015).

17.9 Role of Valuable Bacteria in Animals Production

Bacteria have been recognized as group of biotechnological tools that can enhance the level of immune system and functional gastrointestinal tract in animal. Typical examples of the function of such bacteria in the animal include production of short-chain fatty acids (SCFA), immune development, vitamin supplementation, pathogen exclusion, and compound detoxification (Hooper et al. 2003; Savage 1986; Stevens and Hume 1998).

It has been documented that more than 400 bacterial species are available in the intestinal community in humans (Eckburg et al. 2005), while the application of molecular techniques has validate the presence of 240 species bacteria that are present in the intestine of chicken (Zhu et al. 2005). It has been observed that there higher level of stability in the mature intestinal microbial populations, but the presence of antibiotics and the introduction of pathogenic microorganisms could alter the microbial composition and their population (Zoetendal et al. 1998; Donovan et al. 2002; Muscato et al. 2002).

Intestinal microflora play a crucial role in the metabolism of feedstuffs and possess a greater influence on the rate of animal digestion. It has been observed that microbial metabolism in the intestine could lead to generation of fermented products such as SCFA, which are also regarded as volatile fatty acids, predominantly formed butyrate, acetate, and propionate (Bergman 2002).

It has been observed that microbial-derived SCFA are transported and absorbed through the help of host epithelia, and they are normally applied for cellular energy sources and play a crucial role in the differentiation and colonocyte formation differentiation (Titus and Ahearn 1988). Moreover, some animals most especially the ruminant animal pretends the capacity to obtain a reasonable amount of daily energy requirements from microbially derived SCFA (Stevens and Hume 1998). They perform a lot of functions in the rumen such as SCFA absorption, fermentation of consumed feed, and microbial degradation (Russell and Hespell 1981).

Intestinal microflora also perform several functions in the metabolism in non-ruminants and gastrointestinal function. An experiment was performed where traditionally raised mice with indigenous microbiota possess 42% more body fat when compared to mice breed under germ-free conditions. It has established that the colonization of the germ-free mice with fecal microflora derived from 57% increase in total body fat within 2 weeks without considering the diet intake. It was observed that intestinal colonization of germ-free mice led to altered host expression of fasting-induced adipose factor, a lipoprotein lipase suppressor that is accountable for triglyceride-derived fatty acid uptake and storage by adipocytes and enhanced carbohydrate uptake (Backhed et al. 2004).

17.10 Conclusion and Future Recommendation

This chapter has provided a detailed information on the direct-fed microbials and mechanism of action of direct-fed microbials, while the information were also provided on the significance of biotechnology in feed enhancement such as refining nutritive value of cereals, eliminating anti-nutritive factors from present in feeds, metabolic modifiers, improving the process of digestibility of low-quality forages, and increasing the role of rumen. Detailed information were also provided on beneficial microorganisms that are used in commercial production of animal feeds most especially when yeast, fungal, and bacteria are applied in the production of animal feeds. The application of nanotechnology could also play a significant role in the production of more nutritionally enhanced animal feeds. The screening and identification of more novel enzymes need to be carried out that could facilitate the process involved in the biodegradation of cellulose material that are applied in the development of animal feeds. They could also function in the process involved in the nutritional enhancement of the raw material that will be processed into animal feed.

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Chapter 18
The Link Between Animal Manure and Zoonotic Disease

Shamik Polley, Swaraj Biswas, Shyam Sundar Kesh, Apratim Maity, and Subhasis Batabyal

Abstract Animal manure consists of mainly urine and faeces; additionally, it also contains bedding materials, leftover feed, cleaning water, and other farming wastes. Manure is a good source of plant nutrients which is typically applied to soils as fertilizer by farmers to improve the fertility of the soil. Domestic livestock produces a huge amount of manure annually and is utilized for several purposes and eventually reintroduced into the environment. Livestock manure is a well-recognized potential source of a wide variety of infectious agents, for instance, bacteria, protozoa, and viruses which are responsible for outbreaks of many zoonotic diseases in humans and animals, directly or indirectly, particularly through consumption of contaminated water or food. Excess use of various antibiotics and drugs as preventive medicine in livestock farms also introduces antimicrobial resistance genes into the environment. Livestock manure acts as a source of infection for various zoonotic diseases when it is used fresh or untreated on agricultural land, and consequently it contaminates surface water as well as the environment. Livestock manure is a valuable agricultural good, but proper storage, treatment, and handling of manure are very much essential to minimize pathogen survival and spread in the agricultural production system. Thus, improved and updated knowledge of pathogens shed in livestock manure capable of causing zoonotic disease is needed to identify and control zoonoses which are gradually becoming diseases of public health importance.

Keywords Manure · Livestock · Zoonotic · Bacteria · Protozoa · Virus · Fertilizer · Public health

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_18

18.1 Introduction

Since the last century, monitoring infectious diseases which are predominantly emerging and re-emerging in nature has been challenging. Better quality nutrition, hygienic practice, and development of numerous vaccines and antibiotics reduced the infectious disease burden in the human population in recent decades. Pathogens that infect both humans and animals are commonly called zoonotic pathogens. Despite modern healthcare systems, zoonotic pathogens are liable for the majority of new human disease terrorizations and numerous recent international epidemics. At present, changes in human activities, for instance, growing trade and travel, expansion of human and livestock populations, human food habits, agricultural practices, animal husbandry, and rearing of pet and wild animals, have been linked to a rise of many zoonotic diseases (Morse 1995; McMichael 2004; Harper and Armelagos 2010). The previous report based on analysis of human pathogens stated that 58% of species of human pathogens were zoonotic and 13% were emerging (Woolhouse and Gowtage-Sequeria 2005; Cleaveland et al. 2001).

Human population expansion followed by the development of modern agriculture promoted encroachment into wildlife habitats, and subsequently, both humans and livestock were exposed into closer proximity to wildlife and vectors of potentially zoonotic pathogens. The modified wildlife population reduces biodiversity and creates unique opportunities that favour particular hosts and vectors for spillover of unknown pathogens into livestock, poultry, and humans (Jones et al. 2008; Maudlin et al. 2009; Childs et al. 2007).

The effect of the agricultural transition on human populations is directly associated with population expansion; unfortunately, this incident leads to the emergence of several human contagious diseases, many of which are of animal origin. Migration and colonization of diverse human populations accelerated the disease exposure to naïve populations. The transition from small hunter-gatherer to agricultural societies was linked with variations in human disease load, spatial distribution, and pathogen types of infectious diseases.

The oldest organic fertilizer used in agriculture is animal manure. Animal manure has been an integrated part of sustainable crop production since the beginning of human agricultural activities over 8000 years ago in Neolithic Europe (Bogaard et al. 2013). Animal manure represents universal fertilizer and is constituted of animal faeces and urine. In addition, it also contains straw used as livestock bedding, additional water, and wasted feed. It is a good source of organic matter which improves soil quality and comprises a wide range of nutrients such as nitrogen (N), phosphorus (P), and potassium (K), copper (Cu), manganese (Mn), and zinc (Zn). Animal manure can be applied to soil year-round and the chemical composition of animal manure depends on several factors such as animal species, diet, digestibility, protein, fibre percentage, age of the animal, housing design, environmental condition, and production stage of the animal.

Applications of animal manures to soil increase soil organic matter and improve soil physical conditions, even though hazardous materials such as heavy metals and pathogens potentially pollute the environment. Around the world the most commonly used animal manure in the agricultural field are cattle. However, other livestock manure, for instance, horses, buffalo, sheep, goat, turkey, and rabbit, are also used in different parts of the world. Animal manure commonly carries a wide range of microorganisms which can be classified as pathogenic and non-pathogenic for both animals and humans (Godwin and Moore 1997). The pathogenic loads and varieties of pathogens found in manure depend on species of animals, dietary composition, animal health status and developmental stage of the animals, physical and chemical characteristics of the manure, and storage of the manure (Hutchison et al. 2005; Spiehs and Goyal 2007).

The survival of different pathogens in the manure depends on several factors like types of the pathogen, chemical composition of manure, source of animal manure, co-existence of pathogens, moisture content of manures, and several other factors. The animal manures are either to be stored or disposed of properly; otherwise, they may infect both animals and humans either directly or indirectly (Pell 1997; Hess et al. 2004; Martin 2005). Various potential zoonotic pathogens with the greatest risk of infection for humans are commonly found in manure of different livestock species and poultries. The potential pathogens commonly found in animal manure are bacteria, protozoa, and viruses.

Recent clinical and epidemiological research is mainly concerned with the zoonotic transmission of respiratory and vector-borne pathogens, for instance, Ebola and West Nile virus, while not as much attention has been given to the role of contact with animal faeces/manure in causing human illness (WHO 2006). In low- and middle-income countries (LMICs) livestock and poultries are commonly reared in the domestic environment, and individuals in these countries have regular interaction with animals (Dione et al. 2011; Zambrano et al. 2014). As a consequence, the transmission of zoonotic pathogens from animal faeces contributes to a substantial burden of disease in humans. The maximum amount of manure mainly accumulates from poultry, cattle, sheep, and pigs followed by humans and other animals. Inappropriate storage and disposal of animal manure in the environment are liable for the spread of zoonotic pathogens from animals to humans through faecal-oral transmission (Food and Agricultural Organization FAOSTAT 2017).

Till now sufficient information on zoonotic pathogens in livestock and poultry manure is not sufficient enough to understand the burden of disease from them. Comprehensive and in-depth knowledge of zoonotic diseases linked with livestock and poultry manure is essential for forecasting, noticing, and monitoring these zoonotic diseases in the future.

18.2 Different Types of Animal Manure

The quality of animal manure is influenced by livestock and poultry species. Usually, manures of domestic livestock are applied to the agricultural field to improve the crop yield together with the fertility of the soil (Hepperly et al. 2009).

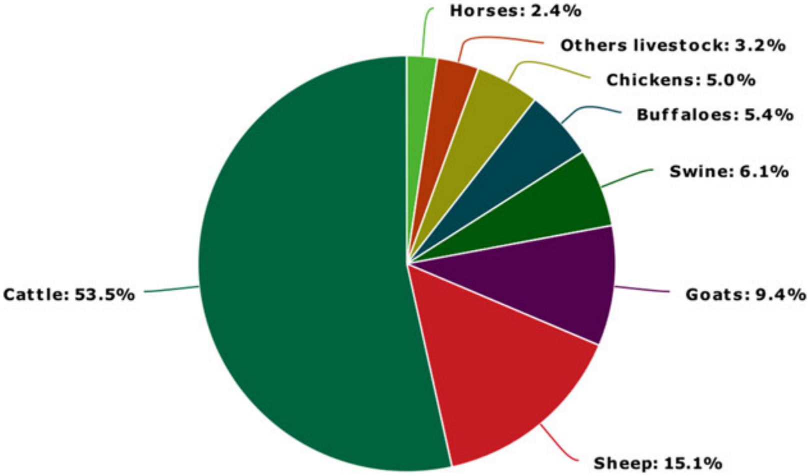

Fig. 18.1 Total amount of nitrogen input of animal manure excreted by different livestock species in the world (1961–2018) (Data source: FAOSTAT Domain Livestock Manure, 2020)

Animal manure must compost adequately and safely for at least 6 months or longer before spread on agricultural fields and gardens. Proper manure management practices must be applied to minimize the transmission of zoonotic pathogens from manure to livestock and human. The proportion of livestock manures obtained from different livestock species all over the world (FAO 2020) is described here (Fig. 18.1).

18.2.1 Chicken Manure

Chicken manure is the best kind of manure for its land application as an organic fertilizer. It contains an excessive quantity of nitrogen and is very good in potassium and phosphorus (Enticknap et al. 2006). Chicken litter is contaminated with various pathogens, including bacteria, fungi, helminths, parasitic protozoa, and viruses, antibiotics and antibiotic-resistant genes, growth hormones such as egg and meat boosters, heavy metals, and pesticides which are dangerous to livestock, human, and environmental health (Griths 2007).

18.2.2 Cattle Manure

Cattle manure is applied to enrich the soil with different macronutrients, such as nitrogen (N), phosphorus (P), potassium (K), sulphur (S), and magnesium (Mg) along with different trace elements (Bernal et al. 2009). Application of both raw cattle manure and composted manure directly onto land as fertilizers helps to boost crop productivity and improve soil fertility for a longer period than synthetic fertilizers (Hepperly et al. 2009). However, raw cattle manure should be avoided as it poses threat to both human and animal health due to the presence of traces of antibiotics, heavy metals, and potentially zoonotic pathogens (Leclerc and Laurent 2017).

18.2.3 Buffalo Manure

Buffalo dung is a good source of macronutrients such as nitrogen (1.03–2.12%), phosphorus (1.30%), and calcium (2.23–5.54%) (Pindozzi et al. 2013). Besides containing nutrients, buffalo dung comprises cellulolytic, hemicellulolytic, and amylolytic bacteria that play a role in the hydrolysis of organic matter (Campanile et al. 2010). During the composting process of organic waste, the buffalo dung waste is commonly applied as a bio-activator for the composting process (Anwar et al. 2021). Application of buffalo manure directly to fields as fertilizer poses risk for zoonoses as it carries several potential zoonotic pathogens.

18.2.4 Horse Manure

Horse manure is enriched with sufficient amounts of organic matter to consider a good fertilizer. Compost horse manure is considered an inexpensive fertilizer for the vegetable garden to improve soil fertility. The deleterious effect of salinity present in soil can be prevented by horse manure fertilizer. In addition, it improves the water holding capacity of the soil (Wheeler and Zajaczkowski 2001). Horse dung poses a potential risk of phosphorus leaching into the environment (Saastamoinen et al. 2020).

18.2.5 Sheep Manure

Sheep manure is a natural slow-release fertilizer and is commonly known as cold manure as a result of its low nitrogen content. The application of sheep manure increases soil organic matter and soil cation exchange capacity; as a result, it improves the nutrient retention capacity of the soil (Nightingale et al. 2004). The application of sheep manure increases the uptake of soil available nitrogen and improves the nitrogen status of plants (Shufu and Huairui 2004). It is a good source of both phosphorus and potassium which help plants to establish strong roots as well as pests defend (Dong et al. 1997).

18.2.6 Goat Manure

Goat manure is most commonly a fertilizer for direct applications to flower and vegetable gardens. It is a dry pellet and less messy than other manures which is easy to collect and apply in the field. Goats manure is odourless and doesn't typically attract insects. The goat manure covers ample amounts of the nutrients of blossoming plants. Application of goat manure increases soil microbial biomass phosphate and improves phosphate fertilizer effectiveness through enhanced biological cycling of phosphorus (Gichangi et al. 2010). The application of goat manure in flower and vegetable gardens contaminated with zoonotic pathogens like *Brucella abortus* represents a potential risk for animal and human health (Wallach et al. 2008).

18.2.7 Pig Manure

Application of pig manure introduces the reoccurrence of organic matter and other nutrients to the soil. It increases fertility along with the moisture-holding capacity of the soil. It is an excellent source of both macronutrients like nitrogen (N), phosphorus (P), and potassium (K) and a range of micro-nutrients including zinc (Zn), sulphur (S), copper (Cu), magnesium (Mg), manganese (Mn), iron (Fe), calcium (Ca), and chloride (Cl) (Pirlo et al. 2016). Pig manure aerates the soil and maintains moisture in soil which is advantageous for the optimum growth of the plant. Still, various zoonotic pathogens (*E. coli*, *Salmonella*, etc.) of pig manure exhibit a potential threat to animal and human health (Jensen et al. 2016).

18.3 Manure, Risk of Zoonoses, and Modes of Infection

The excreta (faeces and urine) of livestock, poultry, wild animals, and birds are widespread across planet Earth. Livestock manure comprises drinking and cleaning water and discharges from different body parts or glands like the nose, throat, vagina, mammary glands, and skin and blood (Pell 1997). Animal manure is used as a good quality of fertilizer in the agricultural field since the domestication of animals and birds. Manure reintroduces organic material and nutrients to the soil for sustaining or improving the quality and fertility of the soil (Hutchison et al. 2000). Additionally, animal dung cake and biogas manufactured from manure are used as fuel for cooking and heating (Venkataraman et al. 2005; Amon et al. 2007).

Human exposure to potential zoonoses increases because of inappropriate clearance of animal excreta and improper handling and use of manure for many applications of human civilization. Contaminated manure may infect humans via direct exposure through the handling or processing of manure; additionally contaminated

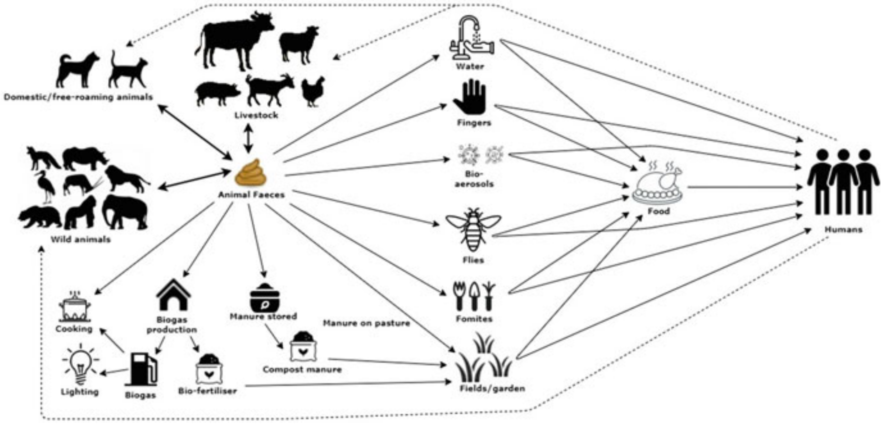

Fig. 18.2 Schematic representation showing the impact of human exposure to animal manure and potential faecal-oral transmission routes of zoonotic pathogens from animal manure to humans

meat, milk, or water may spread zoonotic pathogens via an indirect route of transmission. Animal manure contaminates meat and meat products during the slaughtering and processing of animals. Livestock manure containing zoonotic pathogens may pollute water and animals, or human may be infected through the contaminated water (Millner 2009). Persons directly related to either animal husbandry or manure processing are highly susceptible to zoonotic infections linked with animal manure. Livestock farmworkers' exposure to bio-aerosols generated within animal housing facilities from manure, feed, or the animals themselves leads to a higher risk of zoonotic infection (Pillai and Ricke 2002). Farmers and agricultural workers can be infected through bio-aerosols produced during mechanical spreading of manure in the agricultural field as a fertilizer, and animal manure also contaminates environment, water, or crop (Hutchison et al. 2008) (Fig. 18.2).

18.4 Pathogenic Microorganisms in Animal Manure

18.4.1 Bacteria

Many different bacterial species were screened after exploring their potential to impact animal and human health. The potential bacterial pathogens in animal faeces are *Escherichia coli, Salmonella* spp., *Mycobacterium* spp., *Bacillus anthracis, Brucella* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Chlamydia* spp., *Leptospira* spp., *Rickettsia* spp., *Clostridium* spp., *Yersinia enterocolitica*, *Klebsiella* spp., etc. (Mawdsley et al. 1995). In the absence of proper sanitary measures, direct and food- or waterborne zoonotic transmissions of these pathogens are quite common. Among these, the incidence of some bacterial species, namely, salmonellosis, *E. coli* O157:H7, and listeriosis, has increased in recent years and has become a greater public health concern (Strauch 1991).

The significance of *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter* spp., *Mycobacterium paratuberculosis*, *Yersinia* spp., *Enterococcus* spp., *Bacillus* spp., and *Clostridium* spp. concerning their role in causing outbreaks from the contamination of animal faeces/manure is discussed below.

18.4.2 E. coli

Enteropathogenic *E. coli* (EPEC) is responsible for a severe form of persistent, watery, and/or mucus-containing diarrhoea that is very difficult to treat using routine rehydration protocol (Croxen et al. 2013). Cardinal signs of this type of diarrhoea involve fever, vomiting, and dehydration (Croxen et al. 2013; Heymann 2015). Globally, close to 12,000 diarrhoea deaths per year can be attributed to EPEC, and children below 5 years old are mostly affected (Wang et al. 2016).

Atypical EPEC (aEPEC) and typical EPEC (tEPEC) are the two variants of enteropathogenic *E. coli*. Both differ in their severity of illness and route of transmission. Cattle, sheep, pig, dog, rabbit, and non-human primates are described as animal reservoirs of aEPEC, whereas there is no report of animal involvement in the transmission of tEPEC, and it is only transmitted by humans (Croxen et al. 2013; Vasco et al. 2016). A study in Ecuador confirmed transmission of aEPEC among children and animals (dog, pig, and chicken), though the children were largely asymptomatic (Vasco et al. 2016).

E. coli O157:H7 is considered one of the most dangerous strains of bacteria as it produces verotoxins; in addition, it can survive in harsh conditions. The verotoxins are associated with varieties of symptoms that include the GI system (profuse bloody diarrhoea, known as haemorrhagic colitis), renal system (bloody diarrhoea leading to renal failure, known as a haemolytic uremic syndrome), and the nervous system (involvement of central nervous system along with haemolytic uremic syndrome, known as thrombocytopenic purpura) (Pell 1997).

E. coli O157:H7 remains sustainable in animal manure for an extended period in hostile conditions. It can survive at 5 °C, 22 °C, and 37 °C for 70, 56, and 49 days, respectively, with an initial inoculum of 105 cfu/g (Wang et al. 1996). In the beginning, the mesophilic digestion of manure resulted in a fast decline of viable *E. coli* count followed by a persistent population of *E. coli* for a prolonged period (Kearney et al. 1993).

The feeding practice of cattle is beneficial for the proliferation of *E. coli* O157:H7 in their rumen. The GI tracts of undernourished cattle are advantageous for the multiplication of *E. coli* O157:H7 in contrast to the well-fed cattle which have the greater volatile fatty acid (VFA) concentrations and lesser pH in their gut. Thus, the excretion of *E. coli* O157:H7 can be controlled by the fermentation process in the rumen and hindgut of the animal.

In the abattoir, before slaughtering the animals, providing adequate feed minimizes the chance of contamination at the meat processing plant. The survey executed in cattle farms with well-fed cattle may produce a false-negative result for the prevalence of *E. coli* O157:H7 (Rasmussen et al. 1993). The weaned calves less than 24 months old shed *E. coli* O157:H7 more vigorously than the milk-fed calves, while mature cows are less likely to excrete the same. Earlier studies also reported that the shedding of the pathogen by any particular animal was not uninterrupted and rather intermittent (Rasmussen et al. 1993).

18.4.3 **Listeria** ***spp.***

Listeria monocytogenes is a Gram-positive, rod-shaped bacterium that is very common in the environment. Symptoms associated with infection of this organism may include abortions, septicaemia, and encephalitis (Sobsey et al. 2006). *Listeria monocytogenes* can grow at a broad range of pH ($\leq$5.5–9.0), temperature (3–42 °C), and elevated salt concentrations (up to 12%) that make its control difficult (Bille and Doyle 1991). Naturally, *L. monocytogenes* thrive in plant and soil environments, and silage that is poorly fermented often contains a high load of *L. monocytogenes* (Grant et al. 1995; Wiedmann et al. 1996). It has been isolated from mammals (42 species), birds (22 species), fish, crustaceans, and insects (Pell 1997). Mostly it causes severe neurological symptoms in humans which may even lead to death. Healthy animals may serve as asymptomatic carriers of *L. monocytogenes*.

The organism is propagated through the faeces of infected animals with or without any clinical symptoms. Cow, sheep, goat, pig, cat, dog, chicken, duck, pigeon, turkey, pheasant, sparrow, seagull, rat, rabbit, and other wildlife are possible carriers of *Listeria* (Ryser and Marth 1991). The excretion of *L. monocytogenes* is very common in cattle faeces during the winter than during the summer (Bille and Doyle 1991).

There are reports about several foodborne outbreaks concerning the consumption of raw vegetables which were fertilized earlier with sheep dung, as well as infections linked with intake of unpasteurized dairy foodstuffs and with ice cream that had been contaminated during processing (Van Renterghem et al. 1991; Hennessy et al. 1996).

The growth of *L. monocytogenes* can be restricted by reducing the moisture content of manure by 10% through drying followed by ammonia gas exposure. Growth of *Listeria* can also be stopped by treatment with lactic acid. The prevalence of *Listeria* can also be restricted through proper sanitation and hygiene measures, appropriate cooking of meats and meat products, and pasteurization of milk (Sobsey et al. 2006).

18.4.4 Salmonella *spp.*

Salmonella Typhi is the most widely known organism of this group that causes typhoid fever, but other members include *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Salmonella* Dublin. Salmonellosis is known to cause symptoms like nausea, vomiting, diarrhoea, cramps, and arthritis (nearly 2% of the cases) (Food and Drug Administration 1992). Immunocompromised persons, particularly patients suffering from AIDS, are at high risk. In the year 2010, the cases of invasive non-typhoidal *Salmonella* (iNTS) were reported approximately 3.4 million which led to over 650,000 annual deaths half of which happened in Africa (Ao et al. 2015). Domestic animals such as cattle, swine, poultry, and wild animals such as rodents, reptiles, and insects can act as the host for NTS (Hilbert et al. 2012). NTS remains viable in soil and rodent or insect faeces for months as well as grow easily in contaminated food (Lynch and Tauxe 2009; Mitscherlich and Marth 1984). Primarily two serovars of *Salmonella* (Typhimurium and Enteritidis) are found to be accountable for the significant impact on human health (Crump et al. 2015; Hendriksen et al. 2009).

Approximately 45% of the foodborne disease cases are associated with *Salmonella* spp. infection (Losinger et al. 1995). Many of these gastroenteritis incidences have been traced to animal origin foods, including eggs (Tauxe 1991), although several other sources like salad dressing, peanut butter, coconut, fish, and chocolate have been implicated (Food and Drug Administration 1992). Transmission of *Salmonella* spp. through food is mostly responsible for the elevated level of global NTS cases (Majowicz et al. 2010). Outbreaks of *Salmonella* spp. may also arise from contamination of water (Clegg et al. 1983). NTS infection is mostly zoonotic in origin with little involvement of human-to-human transmission (Okoro et al. 2012). Salmonellosis involves economic loss to the tune of $1 billion/yr. (Losinger et al. 1995).

The incidence of *Salmonella* spp. infections was high since the beginning of documentation in 1943 which was further elevated after 1970 (Tauxe 1991). The emergence of antimicrobial resistance among NTS serotypes in recent decades has further complicated the problem and has become public health concern (Crump et al. 2015). Effective NTS vaccine is available in poultry, but the same is yet to be developed for animals or humans (Desin et al. 2013; Gal-Mor et al. 2014).

18.4.5 Campylobacter *spp.*

The most prevalent species-causing infections in human beings are *Campylobacter coli* and *C. jejuni* although *C. ureolyticus* and *C. concisus* are considered as newer challenges to humans (Kaakoush et al. 2015; Delahoy et al. 2018). *Campylobacter* is very much prevalent in poultry and cattle, but other species such as dogs, cats, pigs, rodents, and birds can act as reservoirs of *Campylobacter* too (Heymann 2015). The transmission is predominantly foodborne and waterborne and mostly involves contact to chicken droppings/chicken manure (Butzler 2004; Kaakoush et al. 2015; Pitkänen 2013). Exposure to domestic chicken was strongly linked with *Campylobacter jejuni* infection in Lima, Peru (Grados et al. 1988). Findings from a study in Ecuador revealed transmission of *C. jejuni* across different species like dogs, chickens, rabbits, and guinea pigs as well as children (Vasco et al. 2016). *C. jejuni* persists as an infective pathogen in the chicken litter for several weeks (Ahmed et al. 2013; Kaakoush et al. 2015). *Campylobacter* prefers cold and humid surroundings outside of a host (Wilson et al. 2008).

Approximately 37,500 deaths were reported due to acute diarrhoea of *Campylobacter* origin globally during 2015, and the majority of this mortality (30,900) was reported in the children age group of fewer than 5 years (Wang et al. 2016). It is mainly prevalent in Asia, Africa, and the Middle East, and 5–25% of gastrointestinal cases were documented in low and middle-income countries (Kaakoush et al. 2015). *Campylobacter* infection is accompanied by diarrhoea, abdominal pain, and fever (Butzler 2004). The long-term sequelae of *Campylobacter* infections may lead to reactive arthritis and Guillain-Barré syndrome (Butzler 2004; Wilson et al. 2008).

Hygienic food preparation, proper water treatment, and exclusion of chickens from the household may help reduce the incidence of this infection (Oberhelman et al. 2006). The establishment of clean water facilities for livestock may be a scientific approach to decrease the infection rate in cattle and other livestock species (Ellis-Iversen et al. 2009, Kaakoush et al. 2015).

The occurrence of fluoroquinolone-resistant *Campylobacter* in Asia and Africa in the early 1990s and consequently the appearance of macrolide-resistant clinical isolates direct the appearance of antimicrobial resistance in these specific organisms (Luangtongkum et al. 2009).

18.4.6 Mycobacterium paratuberculosis

M. paratuberculosis causes Johne's disease or paratuberculosis. The infection primarily follows the faecal-oral route of pathogen transmission, and it may be transmitted prenatally as well as postnatally. The noticeable clinical sign and symptoms of the disease mainly appear at the later stage of the infection, and thus the disease control is quite problematic. The pathogen has plenty of time to become entrenched in the herd by the time it becomes apparent that the disease is present. Young calves are more susceptible to *M. paratuberculosis*. Hence, the prevalence of Johne's disease can be reduced if the calf is segregated from the dam at birth before nursing (Rossiter and Burhans 1996). There is enough literature to suggest that the bacteria can move around from the intestinal tract to other organs like the uterus, udder, lymphatic system, and sex organs of bulls. Sexual transmission of the organism is also not very uncommon.

Some scientific literature points towards a controversial linkage between Crohn's disease and *M. paratuberculosis*. Characteristically, Crohn's disease is an infrequent inflammatory response due to the hyperresponsiveness of the intestinal immune system. Recently numerous studies reported that the aetiology of *M. paratuberculosis* and Crohn's disease are interrelated to each other (Chiodini and Rossiter 1996). Weight loss, colic, vomiting, diarrhoea, and constipation are the symptoms associated with this condition. Crohn's disease may also be due to genetic origin (Engstrand 1995). Controlling the rate of bovine *M. paratuberculosis* infection, therefore, will not only help in profit maximization in dairy farms but also be beneficial in terms of reducing the menace of Crohn's disease problem.

18.4.7 Yersinia *spp.*

Yersinia species are distributed worldwide. As far as human health is concerned, *Yersinia* can be classified into two types: *Yersinia* associated with plague (*Yersinia pestis*) and non-plague *Yersinia* species, i.e. *Y. enterocolitica* and *Y. pseudotuberculosis*. Young males are mostly affected by *Y. pseudotuberculosis*, while individuals of all age groups suffer from *Y. enterocolitica* (Butler 1998).

The intestinal tract of human beings is not the normal site of predilection of yersinias. *Y. enterocolitica* infection in human beings causes symptoms like fever, abdominal pain, and diarrhoea. But in immunocompromised patients and if the body is overloaded with iron, it may even lead to sepsis (Bottone 1997). The study revealed that relatively high doses of the bacteria (109 organisms) only can end up with a successful infection in adult human volunteers (Morris and Feeley 1976). Although the symptoms of *Yersinia* infection prevail for 1–3 weeks, the shedding of the bacteria can continue for several weeks after the symptoms have subsided (Marks et al. 1980). Swine and other animal faeces can be the sources of *Y. enterocolitica*. Pig, sheep, goat, cattle, horse, dog, cat, rabbit, rodent, chicken, and waterfowl serve as the major reservoirs for *Y. enterocolitica* (Bottone 1999). Turkey, duck, geese, pigeon, canaries, and pheasant, in addition to the aforementioned species, can also play the same role in the case of *Y. pseudotuberculosis* (Butler 1998). *Y. pseudotuberculosis* infection is self-limiting in humans and involves appendicitis-like syndrome and fever.

Yersinia infection seldom produces any symptom in animals, although the disease has been documented in dogs and sheep and mastitis in cattle. Intra-species transmission in human beings is not significant, even though the bacteria are shed for quite a long time. There are both pathogenic and non-pathogenic *Y. enterocolitica*, but only the pathogenic strains create a threat to human health. They are widely distributed in the environment, and consequently, it is important to differentiate pathogenic from non-pathogenic *Y. enterocolitica*. Pathogenic strains are endowed with chromosomal and plasmid virulence factors that enable them to cause human disease (Sobsey et al. 2006). *Y. enterocolitica* identified in stream water has been tracked back to swine farms. DNA fingerprints of these stream water isolates are identical to the swine faeces isolates collected from a local farm (Pilon et al. 2000).

Even though the mode of transmission is mainly foodborne, there are instances of waterborne outbreaks (Schiemann 1990).

Antibiotics are not that effective against *Y. enterocolitica*, but antibiotic therapy does help to diminish the severity of illness in grown-up children. Below the age of 6 years, antibiotics are not helpful (Hoogkamp-Korstanje and Stolk-Engelaar 1995; Pham et al. 1991). Moreover, there is the arrival of serotype-dependent antibiotic resistance.

Containment of the outbreak depends on successful sanitation measures in the absence of an effective vaccine. Prescribed preventive measures comprise complete cooking of meat in the home and restricting abattoir personnel from exposure to the oral cavity or intestinal contents of slaughter animals.

Yersinia can be isolated from clinical, environmental, and food samples by cold enrichment and then grown in selective or non-selective broth media. Subsequently, it can be plated on Yersinia-selective agar media for colony isolation. The isolates then can be subjected to serological, biochemical, and virulence factor characterization. The isolates cannot withstand temperatures greater than 440 °C and pH lesser than 4 (Mitscherlich and Marth 1984).

18.4.8 **Enterococcus *spp.***

Enterococcus species are Gram-positive, facultatively anaerobic, lactic-acid producing, commensal bacteria that live in the gastrointestinal tract of both humans and animals (Silva et al. 2012). They are spherical and occur either singly, in pairs (diplococci), or as short chains. They are difficult to distinguish from streptococcus physically (Graves et al. 2009). *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus mundtii*, *Enterococcus avium*, *Enterococcus gallinarum*, and *Enterococcus casseliflavus* are some of the members in this group, and they display considerable differences in their virulence, antibiotic resistance, and distribution in fresh and dry cattle manure (Weaver et al. 2005). Usually, enterococci are avirulent and harmless, yet *E. faecalis* and *E. faecium* are known to cause nosocomial infections in humans (Morrison et al. 1997). *E. mundtii* is most frequently reported in cattle manure (Soupir et al. 2006). Enterococci and *E. coli* are regarded as indicator organisms for the determination of the sanitary quality of recreational waters as per the United States Environmental Protection Agency recommendations (Manyi-Loh et al. 2016).

18.4.9 **Bacillus *and* Clostridium**

Bacillus and *Clostridium* species are included in the phylum *Firmicutes* but belong to the classes *Bacilli* and *Clostridia*, respectively (Girija et al. 2013). They are frequently found in cattle manure and are capable of forming spores. The organisms generally form spores and remain dormant when growth conditions are not favourable but readily become vegetative once conditions are conducive. The spores are resistant to disinfectants, desiccation, and heat to a large extent (Manyi-Loh et al. 2016). A study by Marañón and colleagues suggested that pasteurization can reduce the numbers of these organisms in cattle manure (Marañón et al. 2006).

18.4.10 **Bacillus** ***spp.***

Several different *Bacillus* species, namely, *B. anthracis, B. subtilis*, *B. thuringiensis*, *B. cereus*, etc., have been identified in manure. These are, by and large, harmless Gram-positive rods, form aerobic spores, and can remain viable for years in the soil. Unlike others in this group, *B. anthracis* can cause a very serious life-threatening disease called anthrax (Bravata et al. 2007).

18.4.11 ***Clostridium*** **spp.**

This class of bacteria comes under Gram-positive anaerobic spore-forming bacteria that are abundantly present in the gastrointestinal tract (Girija et al. 2013). They can be subclassified into two groups depending on their ability to enter and proliferate within living tissues. The first group consists of *Clostridium tetani* and *Clostridium botulinum* that have hardly any ability to invade and replicate in living tissues, though they are capable of releasing powerful toxins. The second group comprises *Clostridium chauvoei*, *Clostridium haemolyticum*, *Clostridium septicum*, *Clostridium perfringens*, *Clostridium difficile*, *Clostridium sordellii*, and *Clostridium spiroforme* that can invade and grow in living tissues as well as the intestines of the host animal. They make comparatively less powerful toxins than the previous group (Bagge 2009). These organisms are the causative agent of a variety of illnesses like mastitis, blackleg, haemoglobinuria, malignant oedema, and infant botulism in cattle and humans, respectively (Manyi-Loh et al. 2016).

18.4.12 ***Protozoa and Parasites***

A plentiful number of protozoa and parasites excrete through animal faeces. Among them, some have a zoonotic potential and can infect human beings. The most important zoonotic protozoans are *Cryptosporidium* spp. and *Toxoplasma gondii*, and parasites are *Toxocara* spp., hookworms, i.e. *Ancylostoma* spp., *Uncinaria stenocephala*, *Ascaris* spp., and *Strongyloides* spp. These pathogens are primarily transmitted through faeces contaminating environments.

18.4.13 Cryptosporidium *spp.*

Cryptosporidiosis is a zoonotic parasitic disease caused by *Cryptosporidium* spp. It affects a wide range of vertebrate hosts including birds, reptiles, mammals, and most of the aquatic animals caused by 26 recognized species. A joint expert committee of Food and Agriculture Organization (FAO)/World Health Organization (WHO) declared cryptosporidiosis as fifth important disease among 24 most important global foodborne parasitic diseases (O'Donoghue 1995; Xiao et al. 2004; Rossle and Latif 2013; Ryan et al. 2014; Bamaiyi and Redhuan 2017). WHO registered cryptosporidiosis as a globally 'neglected disease' which mainly affects underdeveloped and developing countries (Savioli et al. 2006). Globally cryptosporidiosis is the fourth leading cause of death among children below the age group of 5, with a total of 1.3 million deaths in 2015 (GBD Diarrhoeal Diseases Collaborators 2017). In sub-Saharan Africa, 2.9 million cryptosporidiosis cases occur annually in children less than 2 years of age (Sow et al. 2016).

Animals and humans get infected when they consume food and drink water contaminated with oocysts of the parasite liberated via faeces. The incidence of infection mostly occurs in areas where people do not have access to basic infrastructure or fundamental facilities to avoid drinking water or food contaminated by infectious oocysts in faeces (Burnet et al. 2014). The parasite mainly causes self-limiting diarrhoea in immune-dominant individuals and life-threatening diarrhoea in immunocompromised patients. The infection becomes severe in patients with human immunodeficiency virus infection/acquired immunodeficiency syndrome (HIV/AIDS), individuals under cancer chemotherapy, the young, elderly people, and malnourished children.

The apicomplexan protozoa *Cryptosporidium* spp. are intracellular, however, extra-cytoplasmic parasites that follow a monoxenous (single host) life cycle (Ghazy et al. 2015). The route of transmission is two types, i.e. direct and indirect transmission. Direct transmission occurs through accidental ingestion of oocysts. The transmission occurs between animal to animal, animal to human (zoonosis), human to the animal (reverse zoonosis or anthropozoonosis), and human to human (Hubalek 2003; Xiao and Feng 2008). Human-to-human transmission usually emerges via swimming pools, water playgrounds, hospitals, and daycare centres and during anal sexual contact with human faeces (Guerrant 1997). However, chances of infection can increase with the sexual intercourse behaviour of men who have sex with men *vi*a the faecal-oral route (Pedersen et al. 1996). Direct transmission can also occur through direct exposure to infected animals particularly young calves by veterinarians, veterinary students, animal handlers, or animal researchers who have a high risk of contact with the infected animals.

Indirect transmission can occur through ingestion of contaminated foodstuffs, food materials (foodborne transmission), drinking of contaminated water (waterborne transmission), and fomites such as clothes and footwear that were exposed to the manure of an infected animal or human (Donnelly and Stentiford 2010; Carmena 2010). Transmission via environmental contamination can take place involving the release of human or animal faeces in the environment such as soil, ponds, rivers, sewage, slurry, or wastewater (Xiao et al. 2000; Jiang et al. 2005). Transmission through inhalation of oocysts was reported in children and immunocompromised patients (Ma et al. 1984; Egger et al. 1990). The high prevalence of zoonotic cryptosporidiosis among children has a great concern in African countries. The high prevalence of cryptosporidiosis in Africa might be due to the high positive rate of immunocompromised HIV/AIDS patients, poor sanitary facilities, negligence in the diagnosis of diseases, and economic burden (Abu Samra et al. 2013).

18.4.14 Toxoplasma gondii

Toxoplasmosis is an emerging and re-emerging zoonosis with worldwide public health importance (Muhie and Kebkes 2014). It is an infection caused by a single-cell parasite called *Toxoplasma gondii*. *T. gondii* is a facultative heteroxenous and polyxenous protozoan parasite. *Toxoplasma gondii* was discovered in the little hamster-like North African rodent Ctenodactylus gundi in 1908 (Nicolle and Manceaux 1908). One-third of the world's population is infected by toxoplasmosis (Montoya and Liesenfeld 2004; Ibrahim and Abakar 2015). Toxoplasmosis infection in humans is often unnoticed in immunocompetent people. In the case of primary infection, non-specific flu-like symptoms may appear with lymphadenopathy. The infection turns fatal in the case of congenitally infected foetus and immunocompromised individuals such as HIV/AIDS patients (Remington et al. 2006; McAuley 2014).

Toxoplasma gondii is an intracellular obligate protozoan parasite. It is an opportunistic coccidian parasite that can affect all homoeothermic vertebrates, including human beings (Dubey 2008). The members of the *Felidae* family together with domestic cats are the definitive host of this parasite, while all warm-blooded animals including all livestock species act as intermediate hosts (Martorelli et al. 2019). *T. gondii* exists in nature in three forms, for instance, the sporulated oocysts, the tissue cyst, and the tachyzoite. *T. gondii* has two types of life cycle, i.e. a sexual cycle that completes within the small intestine of the definitive hosts (feline family) while the asexual cycle follows in intermediate hosts (infected animals), including humans. Felidae transmits *Toxoplasma* pathogen through oocysts dissemination which contaminates the environment and acts as a source of infection for other animals including humans (Tu et al. 2018; Calero-Bernal and Gennari 2019).

Domestic cats and wild felines carry *Toxoplasma* infection in latent form. *Toxoplasma* oocysts were identified and isolated from faeces of 17 species of felines which are capable of shedding *Toxoplasma* oocytes in the environment (Dubey 2008; Baneth et al. 2016; Aguirre et al. 2019). One-third of domestic and non-domestic cats carry *Toxoplasma gondii*, and the high density of the domestic cat's population increases the risk of *Toxoplasma* infection (Hatam-Nahavandi et al. 2021). Approximately 30% of the world's human population has anti-*T. gondii* antibodies in their body (Hofmann et al. 2012; Jafari-Modrek et al. 2019).

Sporulated oocysts are very resistant to environmental conditions and remain infective for more than 1 year in favourable environmental conditions (Mancianti et al. 2015). Tissue cysts remain viable in refrigerated (1–4 °C) carcasses or meat for up to 3 weeks and −1 to −8 °C temperature for longer than 1 week, but most of the tissue cysts could not withstand temperature −12 °C or even lower (Tenter 2009). Tissue cysts are killed by raising the temperature to 67 °C and remain infectious at 60 °C for about 4 min and at 50 °C for about 10 min under laboratory conditions. Curing with salt, sucrose, or low-temperature smoking is enough to kill tissue cysts (Kijlstra and Jongert 2008).

Most of the *Toxoplasma* infections in immunocompetent individuals are asymptomatic. In case of symptomatic infection, symptoms show such as fever, lymphadenopathy, non-specific rash, muscle aches, and pains that may last for a month or more. Toxoplasmosis also causes damage to the brain and eyes. Chorioretinitis or ocular toxoplasmosis is a common manifestation in healthy and immunocompromised individuals. It can characterize by focal necrotizing retinitis associated with redness of the eye, reduced vision, blurred vision, and pain in bright light with tearing. Seventy-five percent of infants who are infected in the womb have no such symptoms but may develop symptoms in later life with bilateral eye involvement (Smith et al. 2020).

Toxoplasmosis in pregnant women is associated with fever and swollen lymph nodes. If an immunocompetent mother was infected before pregnancy, there is no risk of foetal infection, but with infections obtained during pregnancy, there is a chance of foetal infection in the womb. The prevalence of congenital infection was 50% and 25% in untreated and treated mothers, respectively. The rate and severity of foetal infection depend on the stages of pregnancy. The infection rate is higher in the second trimester (30%) compared to the first trimester (10–25%); however, severity is also high in both cases. The rate of foetal infection was 50% in the third trimester; however infection becomes subclinical. Toxoplasmosis is also linked with schizophrenia and other psychiatric conditions (Rorman et al. 2006; Moncada and Montoya 2012; Batz et al. 2013).

Toxoplasma infection in immunocompromised patients is due to latent and reinfection. Clinical symptoms are related to ocular infection and CNS involvement. *Toxoplasma*-induced pneumonitis may also occur with symptoms of fever, dyspnoea, and cough. It is diagnosed via bronchoalveolar lavage (Montoya 2002; Robert-Gangneux and Darde 2012).

18.4.15 **Giardia** ***spp.***

Giardia duodenalis (syn. *Giardia intestinalis* or *Giardia lamblia*) is usually found in the faeces of humans, dogs, cats, livestock, rodents, non-human primates, and wild animals (Cacciò et al. 2005). *G. duodenalis* comprises eight assemblages, from A to H, and out of which assemblage A and B are responsible for human giardiasis (Feng and Xiao 2011). Assemblage A is frequently found in different livestock, dogs, and cats. Dogs are also infected by canine-associated assemblage C and D. Human giardiasis is associated with assemblage A subtype AII. This assemblage causes symptomatic giardiasis cases (Cacciò 2015).

It can transmit by faecal-oral route, and humans can be transmitted by faecal contaminated water, food, grounds, or direct contact. Close contact with domestic pets was responsible for *G. duodenalis* infection with assemblage A in Malaysia (Anuar et al. 2014). Dirty floor and chicken rearing are considered risk factors for *Giardia* infection in children below 2 years' age (Rogawski et al. 2017).

The main symptoms related to giardiasis are acute diarrhoea, malabsorption of nutrients, and malnutrition (Anuar et al. 2014; Ortega and Adam 1997) which are correlated with stunted physical growth and persistent diarrhoea (Muhsen and Levine 2012). This pathogen has a limited significance to contribute overall burden of diseases (Delahoy et al. 2018).

18.4.16 Toxocara *spp.*

Pets are responsible for the transmission of different potential health hazardous zoonotic helminths in humans. Domestic dogs and cats harbours nematodes *Toxocara canis* and *Toxocara cati*, respectively, in their intestine (Otranto et al. 2017; Overgaauw and van Knapen 2013) which are responsible for human toxocariasis throughout the globe (Aghamolaie et al. 2018; Ma et al. 2018). Canids, including dogs, foxes, jackals, and wolves, act as a definitive host of *T. canis*; however, cats are the definitive host of *T. cati*. Both nematodes are transmitted through the faecal-oral route. Humans are primarily infected by accidental oral ingestion of infectious *Toxocara* spp. eggs through contaminated soil, i.e. gardens, playgrounds, and sandpits, unwashed hands, or raw vegetables. Humans also can be infected by consuming undercooked meat or offal (chicken, pigs, or ruminants) containing infective larvae and direct contact with pets (Wolfe and Wright 2003; Ma et al. 2018; Strube et al. 2013; Lucio-Forster et al. 2016).

Toxocara eggs released by dogs and cats become infectious in the soil for up to many years under favourable conditions. These eggs contaminate the environment and become a threat to the people in public places. One-fifth of public places are contaminated with *Toxocara* eggs (Fakhri et al. 2018). The presence of *Toxocara* eggs on the fur of dogs and cats may be a risk factor for zoonotic transmission of diseases (Roddie et al. 2008; Bakhshani et al. 2019). The prevalence of *Toxocara* infection in dogs and cats was 11.1% and 17% globally (Rostami et al. 2020). The prevalence of *Toxocara* infection was higher particularly in low-income countries and tropical countries. Worldwide more the 100 million dogs and 118 million cats were infected with *Toxocara*, thus shedding eggs through faeces and ultimately contributing to the public health risk (Rostami et al. 2020).

Four important clinical outcomes of toxocariasis in humans are classified as visceral larva migrans, ocular larva migrans, neurotoxocariasis, and common/covert toxocariasis (Aghaei et al. 2018; Ma et al. 2018; Mohammadzadeh et al. 2018;

Rostami et al. 2019). Ocular larva migrans are the most common syndrome in connection with *T. cati* infection (Fillaux and Magnaval 2013). Viscerally migrating larvae are the most pathogenic in children than adult and morbidities particularly depending on infection intensity, affected organs, duration of infection, age, and immunity of host (Lee et al. 2014; Fan et al. 2015). Symptoms of visceral larva migrans are coughing, fever, eosinophilia, seizures, abdominal pain, respiratory distress, hepatosplenomegaly, weight loss, rash, and anorexia. Ocular larva migrans can cause chorioretinitis and uveitis resulting in eye pain, retinal scarring, decreased visual acuity, and ultimately loss of vision (Ahn et al. 2014).

18.4.17 Hookworms

The most common hookworms of dogs are *Ancylostoma caninum*, *A. braziliense*, *A. ceylanicum*, and *Uncinaria stenocephala* and cats are *A. ceylanicum*, *A. tubaeforme*, *A. braziliense,* and *U. stenocephala* (Prociv 1998). *A. ceylanicum* can zoonotically transmit to humans and develops into adults. *A. caninum* can also reach adult people causing eosinophilic enteritis (Landmann and Prociv 2003). Zoonotic hookworms primarily may cause cutaneous larva migrans (CLM) in humans (Reichert et al. 2016). *A. caninum* and *A. tubaeforme* predominantly reside in warmer climates of dogs and cats. *A. ceylanicum* is found in Asia (India, Thailand, Taiwan, Indonesia, Malaysia, and Borneo), South America (Surinam), and Australia (Traub et al. 2007; Palmer et al. 2007). *A. braziliense* is found in North America, the Gulf of Mexico and the Caribbean Sea, South Africa, Somalia, Democratic Republic of Congo, Sierra Leone, Australia, Malaysia, Thailand, and Indonesia. A. braziliense-like lesions are reported in humans from India, but the morphologic similarity was noticed with *A. ceylanicum* and *A. braziliense* (Thilakan et al. 2007).

The filariform larvae of hookworm from infected animal manure can penetrate the skin of individuals walking barefoot on contaminated soil. At the penetration site, larvae cause an erythematous papular or vesicular rash with different levels of pruritis and severe itching. It is called ‘ground itch’ with a characteristic of a serpiginous track that could grow for weeks to months (Prociv 1998; Upendra et al. 2013). The larva of *A. braziliense* was responsible for cutaneous larva migrans in humans known as ‘creeping eruption’ (Kirby-Smith et al. 1926; Dove 1932; Caumes 2006). In the USA, it is known as ‘plumber’s itch’ as lesion mostly appears on plumbers who work in the moist, shaded, sandy soil. CLM was also reported from beaches with *A. braziliense* endemic area. CLM-like incidence was also reported from India (Mehta and Shenoi 2004). Animal faeces present in the compound were also related to hookworm-related CLM in Brazilian children (Reichert et al. 2016). The Global Burden of Disease Study in 2015 (GBD) estimates approximately 1.8 million DALYs which are due to hookworm infection even though it was not related to human mortality (Kassebaum et al. 2015; Wang et al. 2016).

Sometimes larva of *A. ceylanicum* causes abdominal discomfort in the adult human. It reaches into the human lumen either by CLM or larval ingestion and causes severe eosinophilic enteritis with peripheral eosinophilia and abdominal pain. The larvae were also found in the human colon in Taipei (Landmann and Prociv 2003; Tu et al. 2008).

Cutaneous larva migrans are a self-limiting infection that can be treated with antiparasitic drugs. Preventive measures like wearing protective footwear on grounds and beaches, regular cleaning of animal waste, covering sandboxes, restricting dogs from defecating on public beaches, and washing hands properly should be followed (Houchedez and Caumes 2007; Feldmeier and Heukelbach 2009).

18.4.18 Ascaris *spp.*

Ascariasis is a helminth infection that primarily infects peoples with poor nutritional status. Ascariasis is responsible for one million DALYs and 2700 annual deaths according to the GBD estimates (Kassebaum et al. 2015; Wang et al. 2016). Humans and pigs are the main reservoirs of *Ascaris* spp. The zoonotic importance of *Ascaris* has been grown recently. *Ascaris suum* of pigs can infect humans (Betson et al. 2014). Dogs also can act as a reservoir for human infection (Shalaby et al. 2010). *Ascaris* is transmitted via ova-contaminated soil, either direct contact with farm produce or ingestion (Heymann 2015). Zoonotic transmission of *Ascaris* is more common in developed countries. A recent study suggested that transmission between human and pigs were possible in Europe, Asia, Latin America, and Africa. Ascariasis also causes serious bowel obstruction (Betson et al. 2014).

18.4.19 Strongyloides *spp.*

Strongyloides stercoralis is a nematode that infects humans. It is estimated that around 30–100 million people are infected with *S. stercoralis* globally. But the prevalence of *S. stercoralis* has been underestimated due to difficulties in diagnosis and assessment of infection (Bethony et al. 2006; Engels and Savioli 2006; Schär et al. 2013).

Two *Strongyloides* species, i.e. *S. stercoralis* and *S. fulleborni* can infect human beings. *S. stercoralis* is primarily transmitted by dogs; however, *S. fulleborni* is transmitted via primates. The prevalence rate of *S. stercoralis* is much higher than *S. fulleborni* in humans (Olsen et al. 2009; Schär et al. 2013; Viney 2015). *S. stercoralis* larvae can infect humans through skin penetration. Clinical manifestation is abdominal pain, diarrhoea, skin eruption, and larva current (Olsen et al. 2009; Siddiqui and Berk 2001; Viney 2015). Infection can be life-threatening for immunocompromised patients. *S. stercoralis* can cause hyper-infection which ultimately leads to chronic infection (Engels and Savioli 2006).

18.4.20 Viruses

Livestock and poultry manure carries different viruses, but usually, these viruses are not transmitted directly to humans except influenza viruses in pigs. Any virus that is excreted in the faeces and urine may be transmitted through water and food. Farm animals are known to expel a variety of viruses from their gut. Most of these agents are pathogenic to farm animals, and very few can be communicable to humans through animal manure (Cliver 2009). The important zoonotic virus transmitted through animal manure, directly or indirectly, is the Lassa virus, hepatitis E virus (HEV), and avian influenza A (H5N1) virus (Cole et al. 1999).

18.4.21 Lassa Virus

Lassa virus infection in human being causes Lassa fever. It is an acute viral haemorrhagic illness that particularly occurs in West Africa. The first case of illness was reported in 1969 when a missionary nurse was infected and died in Lassa, a small town in northwestern Nigeria. At first, a nurse was reported to be infected from an obstetrical patient in Lassa, and afterward, two more nurses were infected with similar viral illnesses (Frame et al. 1970; Buckley and Casals 1970). A total of 100,000–300,000 cases with approximately 5000 deaths due to Lassa fever occurs annually, and 58 million people are at risk primarily from West Africa (CDC 2015; McCormick and Fisher-Hoch 2002).

It belongs to the old world arenavirus under the family *Arenaviridae*. Arenaviruses are segmented negative-sense RNA (nsRNA) viruses and are phylogenetically closely related to Bunyaviridae and Orthomyxoviridae. The host of the Lassa virus is 'multimammate rats' (*Mastomys natalensis*) (Bonwitt et al. 2017). The infected rodents can excrete the virus in urine for a lifetime. The rodents are predominantly found in sub-Saharan Africa and numerous in forests and savannas of the west, east, and central Africa. They enter into the houses and food storage area and may infect humans (CDC 2015; Richmond and Baglole 2003; Yun and Walker 2012). People may be infected upon ingestion or inhalation of rodent faeces or by consuming rodent meat. Rodents excrete viruses through faeces and urine. Soiled objects, eating contaminated food, and direct exposure of open cuts or sores with faeces containing infectious viral particles can lead to infection. Transmission of disease by direct contact with a human is also common as the rodents are naturally roaming in and around of household area and scavenge leftover food items. People also get an infection during hunting and preparation of rodent meat. Airborne transmission may occur when small, tiny aerosols are contaminated with rodent faeces inhaled by humans (CDC 2015). Person-to-person transmission is also possible wherever people are exposed to tissue, blood, secretion, or excretion of a Lassa virus-infected person (Richmond and Baglole 2003).

Most of the infections of Lassa fever (about 80%) are asymptomatic or mild with low-grade fever, general malaise, weakness, and headache. In the rest of the cases, symptoms may progress with complications such as haemorrhages in gums, eyes or nose, vomiting, respiratory distress, facial swelling, and pain in the chest, back, and abdomen. The overall mortality rate is only 1%; the mortality rate may cross above 15% in the hospitalized patient with severe presentation (Walker et al. 1982; McCormick et al. 1987). But in the case of an infected pregnant mother, maternal loss or foetal loss is above 85%. Infants (up to 2 years) may suffer from 'swollen baby syndrome'. In the year 2017, World Health Organization (WHO) declared Lassa fever as a priority disease with an increased focus on its endemic potential (World Health Organization 2017a; World Health Organization 2017b).

18.4.22 Hepatitis E Virus (HEV)

Hepatitis E virus (HEV) is a non-enveloped, single-stranded RNA virus mainly associated with large-scale waterborne acute hepatitis epidemics and sporadic infections, especially in developing countries (Khuroo 2008). The transmission of HEV to humans can be done through multiple routes, including parenterally in association with blood transfusion, or vertically, although transmission primarily occurs via the faecal-oral route through contaminated water or the meat of wild or domestic animals (Goens and Perdue 2004; Khuroo 2008). In endemic countries, HEV is reported to account for more than 50% of cases of hepatitis (Dalton et al. 2008). While primarily associated with developing countries and migration within these, autochthonous HEV infections in developed countries are now recognized as a more important source of infection than previously acknowledged (Dalton et al. 2008). The main source for sporadic infection in developed countries is still unclear, although evidence of HEV infection in pigs and antibodies to HEV have been detected in other domestic animals, including cattle, sheep, goats, and horses (Dalton et al. 2008). Studies have shown that 50–90% of pigs are anti-HEV seropositive and that infected animals can release high levels of infectious HEV in their faeces weeks after infection (Teo 2006). The specific mechanisms of transmission of autochthonous HEV infections in developed countries are not known, but it is probable that zoonotic foodborne transmission via manure, especially from pigs, is involved (Lewis et al. 2009).

18.4.23 Avian Influenza Virus

Avian influenza virus (AIV) is a common zoonotic disease mainly in Southeast Asia and also around the world. More than 60 people died in Southeast Asia from the disease since the first case was reported in Hong Kong in 1997 (Yuen et al. 1998; WHO 2005). Avian Influenza survives outside the host body to a certain extent depending on surrounding conditions. AIV is vulnerable to heat and dryness and is easily destroyed by strong acids or alkaline conditions and by disinfectant. In faecal droppings, AIV (H5N2) can be stored at 4 °C for a period of 30–35 days (Beard et al. 1984). The AIV (H7N2) cannot be survived beyond a week in chicken manure at 15–20 °C (Lu et al. 2003). At higher temperatures, AIV (H7N2) can be destroyed easily, and temperature increment is inversely proportional to the survival time of the virus (Department of Primary Industries and Energy 1996). In allantoic fluid or faeces, the survival time of the AIV (H5N1) was 30 min at 33–35 °C (Songserm et al. 2005). The avian influenza virus (AIV; H5N1) was identified in general chicken manure (pH 8.23 and 13.7% humidity) (Chumpolbanchorn et al. 2006).

Generally, wild waterfowl acts as a natural reservoir host of avian influenza viruses. The wild bird can contaminate domestic terrestrial poultry/birds after interaction with them. In domestic poultry and birds, the viruses undertake several mutations and convert to highly virulent strains compared to earlier stains (WHO 2007). Infected birds shed a substantial amount of virus through their manure and contaminate supply water and environment via surface runoff, through groundwater, or via wind dispersal (WHO 2007; Halvorson 2009). The route of transmission of avian influenza H5N1 from poultry to humans has not been fully elucidated, even though recent evidence reveals that infection may transmit via direct contact with an infected bird or by contact with faecal matter through a respiratory or faecal-oral route (WHO 2007). The H1N1 virus spreads between pigs and humans either through natural transmission or via faecal contamination (Halvorson 2009).

18.5 Manure as a Source of Antimicrobial-Resistant Organisms

Livestock manure and poultry litter can also contribute indirectly to zoonotic disease due to the excess usage of antimicrobials and antibiotics in animal husbandry practices. In modern livestock farms, different classes of antibiotics or drugs are used as either growth promoters or prophylactics or are used for the treatment of disease, either at the herd level or in individual animals (Prescott 2008). The prevalence of antibiotic-resistant organism in different livestock and poultry farms around the world poses a zoonotic risk to humans as well as animals and poultries (Gyles 2008).

The antibiotic-resistant bacteria directly infect humans and livestock populations. Sometimes the resistant bacteria act as a reservoir of antimicrobial resistance genes for other pathogenic and non-pathogenic bacteria (Wright 2007).

Pathogenic bacteria acquire antibacterial resistance genes through horizontal gene transfer, and livestock manure is used as a potential source of infection media or environmental contamination by organisms acquiring the antibacterial resistance from these contaminated environments (Walsh and Fanning 2008). Livestock manure contains significant quantities of antimicrobial residues, and the use of manure as fertilizer in the agricultural field increases antimicrobial resistance both in soil and environmental microorganisms (Venglovsky et al. 2009).

18.6 Preventive Measures to Control Zoonosis

Multiple control measures should be implemented for preventing zoonotic infections arising from manure during animal husbandry and crop production. At first, advanced farm practices should be applied to reduce the pathogen burden within the host animal (Doyle and Erickson 2006). The possibility of herd reinfection and human disease can be reduced through the prevention of environmental contamination of manure comprising zoonotic pathogens. Pathogenic organisms can survive and remain infectious in the faecal matter for extended periods (Zhao et al. 2001). Manure of livestock animals and birds acts as a reservoir of bacterial, viral, and protozoan zoonotic organisms (Table 18.1), responsible for numerous zoonotic infections to humans and animals, even though the majority of pathogens of concern are bacteria (Cliver 2009).

Advance hygienic manure management systems, including the composting of manure scientifically for a specific period and treatments of raw manures utilizing physical, chemical, or biological methods, can be implemented in livestock farms to minimize the environmental contamination of manure (Bicudo and Goyal 2003), whereas proper soil management and irrigation practices must be adopted to minimize contamination risk of pathogens in leafy vegetable and fruits (Holley et al. 2008). Stringent control measures should be implemented at food processing facilities to diminish the chance of contamination of products with manure exposure pathogens (Zhao et al. 2001). Types and numbers of pathogens that persist in livestock manure may be varied with animal species, geographical location, and the physicochemical composition of the manure (Bicudo and Goyal 2003).

The practice of good personal hygiene is the best strategy to prevent the transmission of *zoonosis*. Before consumption, raw meat should be cleaned, washed, cooked, heated, or boiled properly, and people should not use or swallow the water of contaminated swimming pools, ponds, or a water park (Rossle and Latif 2013).

Preventive measures should also be taken to reduce the chances of transmission from animals particularly farm animals. Reduction of animal density in the farm, minimizing the contact period of calves with the farm personnel, isolating the infected or susceptible young ones from adult animals, and keeping of short calving

Table 18.1 List of zoonotic pathogen potentially transmitted through animal manure

Name of pathogen	Host	Human infection	Route of transmission
Bacteria			
Escherichia coli	All mammals	Yes	Water and food
Listeria monocytogenes	Cattle, sheep, goat, pig, cat, dog, chicken, duck, pigeon, turkey, rat, rabbit, and wildlife	Yes	Water, food, fomites
Salmonella spp.	Cattle, swine, poultry, and wild animals	Yes	Water, food, fomites
Campylobacter spp.	Poultry, cattle, dog, cat, pig, rodents	Yes	Water and food
Mycobacterium paratuberculosis	Calve and many animals	Yes	Water, food, respiratory
Yersinia spp.	Pig, sheep, goat, cattle, horse, dog, cat, rabbit, rodent, chicken, waterfowl	Yes	Water, food, and direct contact
Enterococcus spp.	Cattle	Yes	Water, food, and direct contact
Bacillus spp.	Cattle, goat; others	Yes	Direct contact, aerosol, Ingestion
Clostridium spp.	Most of the animals	Yes	Food and wound
Protozoa			
Cryptosporidium spp.	Wide range of vertebrate	Yes	Food, water, direct contact
Toxoplasma gondii	Felines; wild felines	Yes	Water, food, contact, direct ingestion
Giardia duodenalis.	Livestock, dogs, cats, rodents, non-human primates, wild animals	Yes	Water, food, direct contact
Helminths			
Toxocara spp.	Dog and cat	Yes	Water, food, contact, Ingestion
Hookworms	Dog and cat	Yes	Direct contact of soil-skin
Ascaris spp.	Swine, Dog	Yes	Ingestion, water, food, soil
Strongyloides spp.	Dog, old world apes and monkeys, cats	Yes	Direct contact of soil-skin
Virus			
Lassa virus	Rodent	Yes	Food, fomites, contact, inhalation
Hepatitis E virus	Swine, rat, chicken	Yes	Water and food
Avian influenza virus	Poultry, wild birds	Yes	Direct contact, droplets

period may reduce the chances of transmission from animals to humans or the environment. Destruction of pathogens in household premises and livestock farms can be managed by heat treatment (dry or moist) or chemical disinfectant like hydrogen peroxide, 5% ammonia solutions, ethylene oxide, and chlorine dioxide. Ozone (O_3) and ultraviolet light (UV light) also have been used to sterilize drinking water (Hoar et al. 2001).

18.7 Manure Management to Reduce the Risk of Zoonosis

Several species of zoonotic pathogens are nurtured by animal manure, and it also acts as a pathogen reservoir for numerous zoonotic microorganisms. Livestock manure plays a real and significant role in the development of various zoonotic diseases. In addition, disease severity may be increased through either direct or indirect exposure to livestock manure. The risk of zoonotic outbreaks posed by manure has increased with both intensive livestock farming and usage of manure in agriculture (Martens and Bohm 2009). Different methods have been developed to decrease pathogens load of livestock manure (Burton and Turne 2003). Livestock manure is treated with chemical, physical, or biological methods as a prophylactic hygiene measure to control or eradicate numerous zoonotic diseases. Treatment of manure is also essential for other purposes, such as ammonia stripping or phosphate precipitation (Martens and Bohm 2009).

Chemical treatment of manure is most commonly applied to control zoonosis during an epidemic. Chemical treatment can be performed for both solid manure and slurries using various chemicals, for example, lime wash, caustic soda, formalin, peracetic acid, and calcium cyanamide (Burton and Turne 2003). Physical treatment of manure is not commonly applied for routine manure management. Physical methods can be employed to disinfect manure using thermal treatment or irradiation. This process is very effective for the control of certain animal disease outbreaks, such as foot-and-mouth disease (Martens and Bohm 2009). Biological treatment of manure is performed with help of different biotechnological techniques. In this method, viable pathogen numbers can be reduced using numerous factors, including antibiosis, pH alterations, redox-potential adjustments, antagonism, nutrient deficiencies, and exothermic metabolism (Martens and Bohm 2009).

The pathogen burden of manure can be reduced after composting manure for a specific period (Bernal et al. 2009). Long-term storage of manure also reduces pathogen load in manure (Duriez et al. 2008); however safe and efficient decontamination of all potential pathogens is not possible by this method (Bicudo and Goyal 2003). Manure management policies and legislation have been developed in various countries to minimize the negative impacts of manure usage (Burton 2009). An appropriate and efficient manure management and treatment system may be implemented after considering many factors, like the physical properties of the manure (solid manure or slurry), types of organisms, the economics of treating the manure, and identifying areas of particular risk (Zhao et al. 2001).

18.8 Conclusion

Raw and composted animal manure is frequently used in the agriculture field all over the world to boost soil fertility followed by increased agricultural production, but it harms animal and public health. We described several important zoonotic pathogens linked with animal manure with their threats to animal and public health. Animal manure not only contaminates agricultural and livestock farms but our surrounding environment. It is very much necessary to implement public health measures before the application of animal manure as fertilizer.

Further research is desirable to explore pathogen survival in different animal manure along with effective disposal methods of zoonotic pathogens. Appropriate storage procedures based on different animal manures are yet to be implemented to minimize the transmission of zoonotic disease. The scarcity of information is available on the true pathogen burden of disease from animal faeces of different livestock origins. Till now sufficient scientific information is not available regarding the specific pathways to expose to animal manure. It is very much necessary to explore all possible routes of human exposure to animal manure along with precise knowledge of animal husbandry and local social and cultural practice to implement an effective control strategy and reduce the subsequent hazard to public health. It is also necessary to investigate different environmental variables which are necessary to implement appropriate storing and removal of animal faeces and manure.

In the immediate future, the use of properly composted animal manure should help reduce the number and range of zoonotic pathogens to reduce the transmission of zoonotic disease from the manure of different livestock species. Advance systems should be developed through multidisciplinary approaches considering different dimensions of zoonotic disease related to animal manure and human practices to reinforce effective controlling of zoonotic disease risk.

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Chapter 19
Feasible Strategies for Enteric Methane Mitigation from Dairy Animals

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Abstract Methane (CH_4), one of the major greenhouse gas (GHG), has a warming potential 25 times greater than carbon dioxide. Agriculture contributes 50% and 60% of all anthropogenic CH_4 and nitrous oxide (N_2O) emissions, respectively, accounting for 10%–12% of total world anthropogenic GHG emissions. Enteric methane impacts significantly to global GHG emissions for approximately 15% of global warming arising from animal agriculture. Ruminants are the most significant emitters of enteric methane among livestock. About 80 million tons of enteric CH_4 are produced annually by livestock around the world. Enteric CH_4 emissions are produced by microbial fermentation of feeds in the rumen, and the lower digestive tract represents a gross energy loss from 2% to 14%. Methane production and emissions from ruminants are rising day by day as the world's ruminant population grows, helping to meet the nutrient demands of the hurriedly expanding human population. Thus, CH_4 reduction in ruminants has emphasized both economic and environmental benefits. Many mitigation strategies are reviewed for CH_4 abatement from ruminant productions, mainly focusing on animal management, diet selection, rumen manipulation, supplementation of fats, organic acids, oils, plant secondary metabolites, dietary feed additives, defaunation, probiotics, etc. These nutritional amendments also improve feed utilization efficiency, so they are more likely to be accepted by farmers. Nevertheless, no sustainable mitigation techniques have been established yet. To significantly reduce methane emissions from ruminants, a combination of diverse CH_4 mitigation methods should be implemented at the farm level. Therefore, a further comprehensive study is needed to investigate proven and effective CH_4 mitigation strategies that are both practical and cost-effective while also enhancing ruminant productivity. This paper reviews current management practices and dietary strategies to reduce CH_4 from livestock production while improving production and focuses on research issues and the necessity for a systems approach to assessing the relative benefits of abatement options.

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S. Mahajan, A. Varma (eds.), *Animal Manure*, Soil Biology 64,
https://doi.org/10.1007/978-3-030-97291-2_19

Keywords Zoonotic pathogens · Organic fertilizer · Bio-aerosols · Berotoxins · Cryptosporidiosis · Toxoplasmosis

19.1 Introduction

Greenhouse gas (GHG) emissions have gained momentum worldwide because of their influence on global warming and climate change. The consequences of GHG emissions on ecological and socioeconomic vulnerability have already been observed, and they will continue to expand regionally and worldwide in the coming years (IPCC 2007). Methane (CH_4), one of the three main GHGs along with carbon dioxide (CO_2) and nitrous oxide (N_2O), has a 28-fold higher global warming potential than carbon dioxide (CO_2) (Pachauri et al. 2014). Ruminants account for around 81% of GHG emissions among livestock, owing to significant methanogenesis by rumen bacteria, which produces 90% of total CH_4 output from ruminants (Hristov et al. 2013; McAllister et al. 2015). About 80 million tons of enteric CH_4 are produced annually by livestock worldwide (Beauchemin et al. 2008). Agricultural emissions of CH_4 account for around 60% of the overall CH_4 from anthropogenic sources, of which 25% ascends from enteric fermentation in livestock (Olivier et al. 2005). The enteric CH_4 produced in the gastrointestinal tract (GIT) of ruminants signifies the greatest direct GHG released by the livestock sector and the largest single source of anthropogenic CH_4 at a worldwide level (EPA 2012). In India, the agricultural sector accounts for 19% of GHG emissions (IPCC 2007), with livestock accounting for 54% via enteric fermentation and 2.7% through manure management (INCCA 2010). Enteric CH_4 emissions, which account for the majority of CH_4 emissions from ruminants, are produced by microbial fermentation of carbohydrates in the rumen and lower digestive tract (Patra 2012). Methane emissions in ruminants represent a gross energy loss from 2% to 14%, depending upon the type of diet (Johnson and Johnson 1995). As a result, reducing methane emissions in animals conserves energy and boosts productivity.

The importance of feeding (milk and meat) the burgeoning population while reducing environmental impacts of livestock production has gained paramount significance over the last decade in developing countries (Golub et al. 2013; Eisler et al. 2014). In the near future, this growth in demand is expected to increase livestock numbers. The number of animals is directly proportional to the amount of CH_4 produced (Table 19.1). In the absence of proper mitigation measures, this

Table 19.1 Livestock contribution to climate change by greenhouse gases emission

GHG	GHG emission (Gt. CO_2 eq./year)	Livestock sector contribution (%)
CO_2	2.0	27
CH_4	3.1	44
N_2O	2.0	29
Total	7.1	100

Source: Tackling Climate Change through Livestock, Gerber et al. 2013 will accelerate the specialization and industrialization of livestock husbandry, exacerbating GHG problems (Steinfeld et al. 2006). It is frequently recommended in developed countries to cull nonproductive and low-producing animals to minimize the CH_4 budget (Patra 2012). They managed to keep high-producing animals in herds to reduce CH_4 emissions per unit of product. On the other hand, this is frequently impractical for developing countries because of their social and religious backgrounds.

It is well established that as animal productivity rises, CH_4 emissions also reduce per unit of product. As a result, there is an urgent need to develop and implement GHG mitigation technologies in livestock production systems. There are several strategies for increasing animal production, including proper diet formulation, protein, and energy supplementation to low-quality forages, ionophores, bovine somatotropin, and probiotics (Moss et al. 2000). Although many CH_4 abatement strategies from ruminants are available (Beauchemin et al. 2008; Cottle et al. 2011; Karakurt et al. 2012; Kumar et al. 2014; Patra et al. 2017; Haque 2018; Banakar et al. 2019), this review summarizes the several methane mitigation options highlighting the new developments in this area and sets up future research needs and identifies obstacles in the mitigation of enteric CH_4 emissions.

19.2 Rumen Microbiome and Methanogenesis

In ruminants and pseudo-ruminants (Camelidae), a significant portion of the methanogenesis happens in the large fermentative chamber known as the rumen. The rumen microbiome is a complex, diverse, and primarily obligate anaerobic microbial ecosystem harboring a wide variety of microorganisms, viz., bacteria (10^{10}–10^{12} ml^{-1}), archaea (10^{8}–10^{10} ml^{-1}), ciliated protozoa (10^{5}–10^{6} ml^{-1}), fungi (10^{4}–10^{5} ml^{-1}), and viruses, staying in a symbiotic relationship within the rumen (Islam and Lee 2018). In the rumen, protozoa can account for up to 50% of the microbial biomass (Newbold et al. 2015). In comparison, the fungi were estimated at around 8% of the total biomass (Orpin 1981). The archaea make up only 0.3%–4% of the rumen microbial biomass (Janssen and Kirs 2008), whereas bacteria make up the rest, which is typically the major component (Tapio et al. 2017). This rumen microbiome plays a pivotal role in feed fermentation, where feeds including fibrous plant structures are fermented primarily to different volatile fatty acids (VFAs), CO_2, hydrogen (H_2), and CH_4. These VFAs are necessary for the ruminant's energy metabolism and protein synthesis (Hungate 1967). Only a few of the diverse rumen microbiomes have been successfully characterized previously using culture-dependent techniques. However, advent in the multi-omics techniques, for example, metagenomics by high-throughput sequencing or next-generation sequencing (NGS) (Fouts et al. 2012; Gharechahi and Salekdeh 2018), metaproteomic (Comtet-Marre et al. 2017), metatranscriptomics (Jami et al. 2014), and metabolomics (Saleem et al. 2013) have been increased significantly.

Table 19.2 Methanogenesis pathways that take place in rumen

Pathways	Reactions	References
Methylotrophic pathway	$CH_3OH + H_2 \rightarrow CH_4 + H_2O$	Boadi et al. 2004; Lovett et al. 2003
	$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	
	$CH_3NH_2 + H_2 \rightarrow CH_4 + NH_3$	
Hydrogenotrophic pathway	$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	Odongo et al. 2007

CO_2—carbon dioxide; H_2—hydrogen; CH_4—methane; H_2O—water; CH_3OH—methanol; CH_3NH_2—methylamine

Methanogenesis is a process in which H_2 reduces CO_2 in the rumen with the help of methanogenic archaea (Ellis et al. 2008). CH_4 formation is the main way for H_2 clearance from fermentation (Hill et al. 2016). The two distinct pathways of methanogenesis in the rumen, carried out by archaea, are presented in Table 19.2. Bacteria, protozoa, and fungi use the hydrogenotrophic route to convert H_2 and CO_2 into CH_4 (Martin et al. 2010). Because most prevalent ruminal microorganisms may use formate that is equivalent to $H_2 + CO_2$, it is usually assumed that formate belongs to the hydrogenotrophic category (Janssen 2010). Another process of methanogenesis is the methylotrophic pathway, which uses methyl groups as a substrate, such as those found in methylamines and methanol (Poulsen et al. 2013). Methanogens have been divided into 28 genera and 113 species, but there are likely to be many more in nature (Janssen and Kirs 2008). From rumen, only seven species have been so far cultured from the rumen (Janssen and Kirs 2008). These are *Methanobacterium bryantii*, *Methanobrevibacter olleyae*, *Methanobrevibacter ruminantium*, *Methanobrevibacter millerae*, *Methnaomicrobium mobile*, *Methanobrevibacter smithii*, *Methanoculleus olentangyi*, and *Methanosarcina* spp. (Janssen and Kirs 2008). Multi-omics approaches have recently been used to understand better greenhouse gas emissions from ruminant production (Wallace et al. 2017).

Unlike methanogens, acetogens use H_2 to generate acetate. In the hindgut fermentation of mammals and termites, they serve as major H_2 sinks. Reductive acetogenesis occurs in the intestine of non-ruminants, which can occur alongside methanogenesis and sometimes replace methanogenesis (Liu and Whitman 2008). Acetogens are the natural rumen flora, yet methanogens outcompete them because methanogens have lower H_2 utilization limits than acetogens, and moreover, methanogens have a thermodynamically more favorable nature than acetogenesis (Attwood and McSweeney 2008).

$$\text{Acetogenesis} : 4H_2 + 2CO_2 = CH_3\,COOH + 2H_2O$$

$$\Delta G^{\circ\prime} = -104.6\ \text{kJ mol}^{-1}$$

19.3 Methane Mitigation Strategies from Ruminants

Methane reduction from animal origin is a time-demanding task all over the world. The world population will reach a benchmark of nine billion by 2050, increasing the demand for animal-origin products (milk and meat) (FAO 2006). At the same time, the total CH_4 emissions from ruminant animals are predicted to increase drastically. Hence, reducing CH_4 emissions from the livestock industry is the need of the hour. Various alternative targets and mitigation techniques have been implemented thus far (Fig. 19.1), but they do not appear to be sustainable. Here, we have compiled a list of the most critical methane reduction strategies used in ruminant production.

19.4 Animal Interventions

19.4.1 Maintaining Low Methane Emitters

The amount of methane generated is directly proportional to the number of animals. In developed countries, the culling of nonproductive and low-producing animals is frequently promoted as a way to reduce the CH_4 budget. Herds of high-producing animals should be maintained together. Even though total production will increase, CH_4 emissions per unit of product will likely decrease. However, given to many developing countries' social and religious backgrounds, this is unlikely to be suggested. Proper livestock management, particularly in developing countries, can reduce CH_4 emissions in a herd for each production unit by minimizing the incidence of diseases and reproductive problems (Eckard et al. 2010).

Recently, the generation of CH_4 for all animals' types and breeds under the same feeding conditions has been examined and shown to vary significantly (De Mulder et al. 2018). Pinares-Patiño et al. (2003) classified some animals as high and low CH_4 emitters based on CH_4 output per unit of feed intake, noting that these differences were maintained over four 5-month assessment periods when the same type of food was given. Although the evidence is inconclusive, it could be owing to differences in methanogen numbers among animals (Zhou et al. 2009). This research shows that there may be genetic variances in CH_4 production among animals, which might be used for the genetic selection of animals with low CH_4 output. Some other studies have demonstrated that ruminants with low residual feed intake (RFI) generate less CH_4 than those with a high RFI. Likewise, Hegarty et al. (2007) stated that CH_4 production was lower in Angus steers selected based on low RFI than in steers having high RFI (142 vs. 192 g CH_4 day^{-1}). Although the reason is not clear, it might be due to variations in methanogen numbers among animals (Zhou et al. 2009). Therefore, this strategy might be a possible and sustainable way for tropical countries to mitigate methane emissions where low-quality feeds are fed to ruminants.

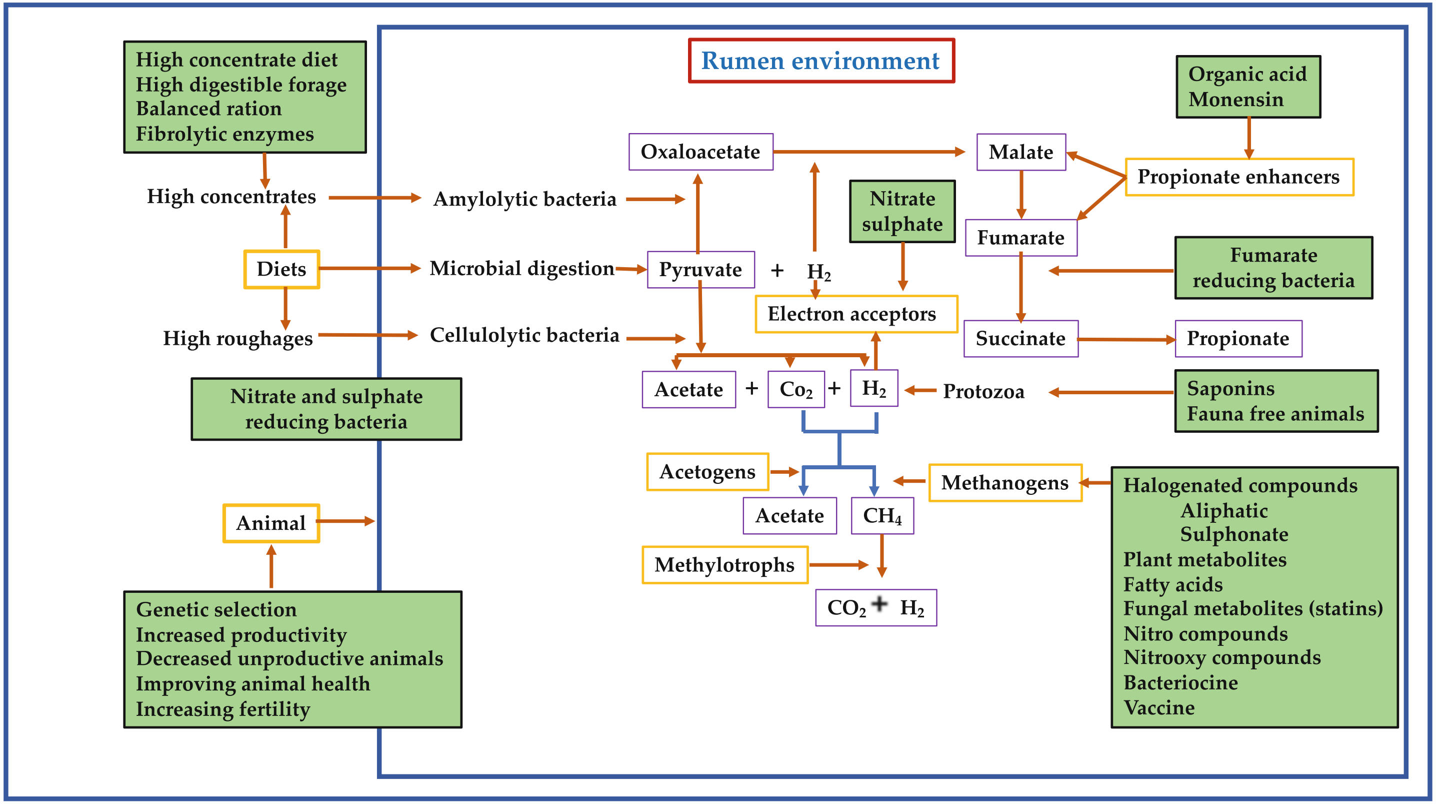

Fig. 19.1 A schematic representation of the possible targets to reduce methane emissions from ruminants. The boxes with dark shade are the strategies that have been studied either in vitro or in vivo to decrease methane production, and the boxes without dark shade could be the targets for methane mitigation (Patra 2012; Islam and Lee 2018)

19.5 Dietary Intervention

19.5.1 A Balanced Ration Reduces Methane Production

The composition of diet influences methane generation in ruminants. Digestion in the rumen is reliant on the activities of microorganisms, which require energy, nitrogen, and minerals (Mwenya et al. 2004). Consequently, the forage quality influences the activity of rumen microbes and CH_4 formation in the rumen. Dietary grain sources and the species, processing, and proportion of forages determine CH_4 generation in ruminants. Methane production tends to reduce as feed protein level rises and increases as the feed fiber content increases (Shibata et al. 1992).

19.5.2 Supplementation of Ionophores, Organic Acids, and Mineral Mixtures

Ionophore antibiotics, such as monensin, salinomycin, and lasalocid, are typically used in ruminants to improve feed efficiency and animal performance. They have also been shown to reduce CH_4 production from ruminants significantly. Ionophores do not change the number or diversity of methanogens, but they do change the bacterial population from Gram-positive to Gram-negative organisms, resulting in a switch from acetate to propionate fermentation. The availability of H_2 for methanogens to produce CH_4 is reduced as a result of this fermentation change. Ionophores might also decrease ruminal protozoal numbers. Compared to doses required to improve feed efficiency, rather large dose levels may be required to reduce CH_4. For instance, studies reported that a high dose of monensin decreased CH_4 production (g/d) by 4%–10% in beef and dairy cattle (Odongo et al. 2007). Consequently, the decrease and subsequent recovery of protozoal counts in the rumen aid in the reduction of CH4 by up to 30% (Guan et al. 2006). Unfortunately, specific long-term experiments suggest that the inhibitory effects of ionophores on methanogenesis may not persist over time as several microbes may get adapted to ionophores (Huhtanen et al. 2015). Conversely, ionophores have been prohibited as feed additives in the European Union and are restricted in several other countries.

The mineral mixture also has profound effects on enteric methane mitigation. For example, dietary supplementation with illite feed additive, a clay-sized mineral mixture containing Mg, Ca, K, Mn, Zn, P, Fe, Al, Si, Co, Se, and Mo, at 1% on a dry matter (DM) basis had a better effect on CH_4 reduction while enhancing VFA production in steers (Biswas et al. 2018).

When ruminants are supplemented with a feed having organic acids such as fumarate and malate, and propionate precursors or compounds, they are potential feed additives that reduce CH_4 emissions (McGinn et al. 2004). They are expected to stimulate enhanced propionic acid production in the rumen by serving as an H_2 sink, lowering CH_4 production in the process (Castillo et al. 2004). According to another study, organic acids can reduce methane production by up to 17% (Newbold et al. 2005). In continuous fermenters employing pasture as a substrate, fumarate reduces CH_4 emission by 38%, according to in vitro research (Kolver et al. 2004).

19.5.3 Supplementation

In developing countries, ruminants are fed low-quality crop residues that are deficient in protein, minerals, and vitamins. As a result of improved rumen fermentation efficiency, dietary supplementation of these low-quality diets with energy or protein supplements could lower CH_4 production. Diets with high concentrations of concentrate feeds produce more propionate, which reduces the amount of H_2 available for CH_4 synthesis. For example, when green sorghum substituted wheat straw by 30%, methane production in crossbred cows reduced by 33% (Haque et al. 2001). The usage of urea/molasses multi-nutrient blocks has been demonstrated to be a cost-effective diet supplementing strategy that can lower CH_4 emissions by 10%–25% while also increasing milk supply (Srivastava and Garg 2002).

19.5.4 Suppression of Rumen Methanogens

Halogenated CH_4 analogs and related chemicals such as chloral hydrate and chloroform have long been studied for their ability to limit CH_4 synthesis in ruminants. However, after a long time of feeding, they induce liver damage and animal mortality. As a consequence, they appear to be unsuitable for usage in practice. 2-Bromoethanesulfonic acid and bromochloromethane, a bromine analogue of coenzyme F implicated in methyl group transfer during methanogenesis, reduced CH_4 outputs, although their anti-methanogenic effect was shown to be transient (Dong et al. 1999). Nitroethane, 2-nitroethanol, 2-nitro-1-propanol, and 3-nitro-1-propionic acid have all been demonstrated to suppress ruminal CH_4 generation in vitro (Anderson et al. 2008), while nitroethane and 2-nitro-1-propanol have also been found to limit CH_4-producing activity in vivo (Gutierrez-Bañuelos et al. 2007). These nitro compounds are intended to function by preventing the oxidation of H_2 and formate (Anderson et al. 2008). Despite the fact that these chemical antimethanogenic agents show potential in reducing CH_4 emissions, research on these chemical feed additives is unlikely to continue due to public concerns over chemical residues in animal products.

19.5.5 Supplementation of Fats

Fat supplementation in the diet reduces CH_4 generation depending on the level of fat supplementation, forms of fat supplementation, fat sources, and diet types. The major substrates for methanogens are carbon dioxide and hydrogen or formate (Moss 1994). Some rumen microbes utilize hydrogen to hydrogenate unsaturated fatty acids with double bonds (acting as an alternative H_2 sink). Thus, the addition of unsaturated fatty acids to the diet reduces CH_4 synthesis (Shibata and Terada 2010). To meet the energy demand of high-producing dairy cows, fat has traditionally been added to the diet to boost dietary energy content. Energy supplementation in ruminant diets has recently shifted from carbohydrate to fat, which contributes to CH_4 mitigation. The mechanism of CH_4 production by fat may be mediated through decreasing organic matter fermentation, fiber digestibility, and the direct inhibition of growth of methanogens and protozoa via the hydrogenation of unsaturated fatty acids in the rumen (Johnson and Johnson 1995). Several other investigations have found that dietary fat supplementation may reduce CH_4 generation from ruminants (Grainger and Beauchemin 2011). Besides, it was also reported that fat inclusion in diets mainly at concentrations above 6–7% of dietary DM often reduces carbohydrate fermentation, particularly fiber components (toxic effects on cellulolytic bacteria and protozoa) and DM intake (Grainger and Beauchemin 2011). As a result, choosing the appropriate fat sources and the right amount of fat supplements is essential. However, the cost of fat supplementation using edible oils may not be economical for animal producers.

19.6 Supplementation of Plant Secondary Metabolites or Botanical Extract

Depending on the sources, type, doses, molecular weight, and diet types, the methane-reducing capability of PSMs varies greatly. Recently, bioactive plant metabolites, viz., tannins, saponins, flavonoids, and essential oils, have potential anti-microbial effects for CH_4 mitigation options (Kamra et al. 2008; Patra and Saxena 2010). These compounds reduce CH_4 synthesis via affecting methanogens directly and/or eliminating protozoa, reducing OM digestion, and modifying rumen fermentation (Patra and Saxena 2010).

19.6.1 Saponins

There is increasing evidence suggesting that supplementation saponins (triterpenoid saponin, methanol extract saponin, tea saponin) in the diets might diminish methane production, which is likely due to a significant reduction of protozoa numbers and/or methanogenic archaeal activity in the rumen (Hristov et al. 2003; Patra and Yu 2013). Saponins from various sources have different effects. Agarwal et al. (2006) found that ethanol, water, and methanol extracts reduced CH_4 generation by 96%, 39.4%, and 20%, respectively, when ethanol, water, and methanol extracts of *Sapindus mukorossi* seed pulps were compared to controls. However, in other in vitro research, an opposite result was found, where Quillaja saponins at 0.6 g/L did not lower the abundance of methanogen or CH_4 generation (Patra and Yu 2015). Though the effects of saponins or saponin-containing plants' methanogenesis and methanogen abundance were found to be transient, they still needed to study more.

19.6.2 Tannins

It has been suggested that tannins exert their effects on methanogenesis by directly inhibiting methanogens and indirectly by decreasing the number of protozoa and fiber digestion and decreased H_2 production in the rumen (Patra and Saxena 2010). Different sources of tannin extracts (condensed and hydrolyzable tannin) have been shown potentials to reduce CH_4 production from ruminants depending upon doses. For instance, supplementation of *Acacia mearnsii* tannin extracts suppressed CH_4 production in cattle up to 30% decreased methanogenesis (Grainger et al. 2009). Likewise, when ruminants were fed tannin-rich forages, CH_4 production was reduced by up to 55% (Ramírez-Restrepo and Barry 2005).

19.6.3 Essential Oils

It contains chemical components and functional groups with antibacterial activity, such as terpenoids, phenolics, and phenols, which impede the growth and existence of many microorganisms in the rumen (Burt 2004). Essential oils are volatile components and aromatic lipophilic compounds that plants produce as secondary metabolites (Tamminga et al. 2007). They have a high affinity for microbial cell membranes due to their lipophilic nature, and their functional groups interact with the microbial cell membrane simultaneously (Jouany and Morgavi 2007). Methanogenesis is reduced with essential oil by reducing microbial populations (Newbold et al. 2004). Ethanol and methanol extracts of *Syzygium aromaticum* and *Foeniculum vulgare* reduced CH_4 production in vitro (Patra et al. 2010). *Allium arenarium* oil (garlic oil), an excellent potential essential oil, drastically reduced methane generation by 12 and 36% both in vivo and in vitro, respectively (Lewis et al. 2013).

19.7 Defaunation

Removal of protozoa known as defaunation is often associated with a higher microbial protein supply and enhancement of animal productivity. Though ciliate protozoa are responsible for up to 37% of rumen methanogenesis (Finlay et al. 1994) and generate a relatively large volume of H_2 and formate, many methanogenic bacteria remain attached to the exterior surface of rumen ciliate protozoa (Ushida et al. 1997). Thus, defaunation is an effective way to decline CH_4 emission with minimal effect on rumen digestion. So far, several defaunation strategies have been tested experimentally, but none are widely utilized due to toxicity concerns for the rest of the rumen microbial population and the host animals (Moss et al. 2000). In recent years, plant secondary metabolites have been investigated as potential defaunating agents. Notably, saponin-containing plants are promising to reduce or eliminate protozoa in the rumen without decreasing bacterial activity (Patra and Saxena 2009).

19.8 New Potential Mitigation Options

19.8.1 Supplementation of Enzyme Additives

Fiber digestibility, feed conversion efficiency, and milk output of dairy cows are all improved by using enzyme feed additives with fibrolytic activity (Rode et al. 1999). Arriola et al. (2011) found a decrease in intestinal CH_4 generation when a fibrolytic enzyme addition was added to a lactating cow's feed (52% dietary forage). On the other hand, according to other research, exogenous fibrolytic enzyme additive enhanced CH_4 output and changed the composition of the rumen methanogen community without changing the overall density of methanogens (Chung et al. 2012). In an in vitro study, Biswas et al. (2016) found that dietary supplementation of lysozyme enzyme improved rumen fermentation and reduced CH_4 emission. Hence, more in-depth in vivo research is required before enzymes may be used for a methane mitigation strategy.

19.8.2 Inclusion of Direct-Fed Microbials: A Biological Methane Mitigating Agent

Modifying the rumen ecosystem using probiotics or direct-fed microbials (DFM) is one promising approach to decreasing CH_4 production in the rumen. Commonly used probiotics in the ruminant's diets include *Lactobacillus*, *Bifidobacterium*, *Megasphaera elsdenii*, *Bacillus*, *Enterococcus*, *Prevotella bryantii*, and *Saccharomyces cerevisiae* (yeast) to improve rumen fermentation, feed efficiency, animal performance, and health status, which could also reduce CH_4 emissions from ruminants as discussed earlier (Chiquette et al. 2007).

19.9 Alternate Hydrogen Sinks

19.9.1 Propionate Enhancers

The generation of VFAs such as acetic, propionic, and butyric acids is mainly determined by the diet given to the animal. Ruminants on a concentrate-based diet produce more propionate than those fed a rich forage diet, producing more acetate. However, when methanogens are suppressed, alternate routes for H_2 utilization should be considered to alleviate the depression of fiber digestion in the rumen. Propionate formation, commonly known as the H_2-utilization pathway, uses reducing equivalents such as pyruvate, which is then converted to propionate (Baldwin et al. 1963). The use of propionate enhancers seems to be promising for the disposal of H_2. Although these H_2 are the most important precursors for the generation of CH_4, an increase in propionate formation is proportionally associated with a decrease in CH_4 generation. The addition of organic acids, where malate, fumarate, and succinate are formed as intermediate products for propionate production through the succinate pathway, may result in a stoichiometric reduction in H_2 availability for CH_4 production. In this pathway, different bacteria such as succinate producers (e.g., *Fibrobacter succinogenes*), fumarate reducers (e.g., *Wolinella succinogenes*), and succinate utilizers (e.g., *Selenomonas ruminantium*) are also involved.

19.9.2 Alternative Electron Acceptors

This class of CH_4 mitigating agents has gained renewed attention in ruminant production systems. Increased H_2 use by organisms other than methanogens could also help to decrease methanogenesis. Some rumen microbes that may convert nitrate to nitrite and then nitrite to ammonia employ hydrogen, formate, or both as electron donors; hence, methanogenesis can be reduced by adding electron acceptors like nitrate and sulfate (Sar et al. 2004). Recent research results show that nitrates inclusion in the diet of cattle decreased enteric CH_4 production by up to 50% (Hulshof et al. 2012).

19.9.3 Stimulation of Acetogens

The reductive acetogenesis is an alternative strategy and accepted mechanism to redirect H_2 utilization from methanogens to acetogens (Joblin 1999). The final product of this reaction, acetate, has the added benefit of providing energy to the animal. Nevertheless, acetogens are less abundant in the rumen environment and less efficient at competing for reducing equivalents than methanogens. Hence, it is necessary to raise the number of acetogenic bacteria in the rumen, which compete with methanogens for hydrogen due to the reduction in CH_4. Lopez et al. (1999) discovered that they suppressed CH_4 generation when acetogens were introduced to rumen fluid in vitro.

19.10 Conclusion

Ruminant production is hurriedly increasing in order to meet the growing demand for high-quality meat, milk, and other ruminant products among the world's population. Methane production is also on the rise proportionally, which has a negative impact on global warming as a greenhouse gas. Therefore, we should focus more on sustainable methane mitigation strategies that can be achieved through a cumulative approach. Various abatement options can be applied in animal production systems either now or in the future, many of which are likely to be cost-effective in their own right. These strategies include animal intervention, supplementation of fats, diet selection, plant secondary metabolites, defaunation, dietary feed additives, organic acids, probiotics, etc. (Table 19.3). Nevertheless, no sustainable mitigation techniques have been established yet. To develop CH_4 mitigation solutions that produce consistent outcomes, much comprehensive research is warranted. Likewise, a broad understanding of both methanogen biochemistry and rumen microbial ecology is needed to achieve CH_4 mitigation effectively.

Table 19.3 Summary of feasible strategies for enteric methane mitigation from dairy animals

Strategy	Potential CH_4 reduction	Technology availability/ feasibility	Cost/production benefits
Improving animal productivity	20–30%	Feasible and practical	Increased feed cost increased milk production use of fewer animals less feed per kg of milk
Increasing concentrate levels at high levels of intake	25% or more	Feasible, for high producing cows, but may increase N_2O and CO_2 emissions	Increased feed intake increased feed cost, machinery/fertilizer use increased milk production
Processing of forages, grinding/pelleting	20–40%	Feasible	Increased cost of processing improved feed efficiency increased milk production
Forage species and maturity	20–25%	Feasible	Increased feed efficiency increased milk production
Rotational grazing of animals/early grazing	9% or more	Feasible	Increased cost of fencing increased management of animals increased feed intake increased milk production
Managed intensive grazing vs. confined feeding		Feasible needs more investigation	Cheaper feed cost may need supplements reduced milk fat/protein content higher net return
Use of high-quality forages/pastures	25% or more	Feasible	Increased feed intake increased milk production
Preservation of forage as silage vs. hay/additives Addition of fats	up to 33%	Feasible and practical, but usage limited to 5–6% in diet	Increased cost of diet increased or no effect on milk production may or may not affect milk fat
Use of ionophores, e.g., monensin, lasalocid	11–30%	Feasible, but not long lasting	Increased feed efficiency decreased feed intake increased milk production
Use of probiotics	10–50% (in vitro)	Feasible, needs more investigation	May increase feed intake may increase milk production or no change
Use of essential oils	8–14% (in vitro)	Feasible, needs more investigation	Not quantified
Use of bovine somatotropin (bst)	9–16%	Not approved for use in Canada	Reduced feed cost
Protozoa inhibitors	20–50% (in vitro and in vivo)	Not available for practical use	Practicability and cost to be assessed

(continued)

Table 19.3 (continued)

Strategy	Potential CH_4 reduction	Technology availability/ feasibility	Cost/production benefits
Propionate enhancers (fumarate, malate)	5–11% (in vitro) up to 23% (in vivo)	Possible microbial adaptation to fumaric acid	Economic feasibility Ruminal adaptation level of inclusion needs to be evaluated
Use of acetogens	Not quantified	Not available, needs more investigation	Needs further investigation
Use of bacteriocins, e.g., nisin, bovicin HC5	Up to 50% (in vitro)	May provide alternatives to ionophores that needs more investigation	Production effects are to be evaluated
Use of methane inhibitors, e.g., BES, 9,10-anthraquinone	Up to 71% (in vitro)	No compounds registered for use No long-lasting effects identified	Increased cost of chemical production effects not established
Immunization	11–23%	Not available, needs more investigation	May increase cost of production increased gain
Genetic selection	21%	Long-term feasibility	Decreased feed intake increased feed efficiency

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