



Methanogens: Way Ahead for Sustainable Development

**Rekha Mehrotra¹, Anantika Singh¹, Angel Merry Indwar¹, Ritika Das¹,
Sonal Roy¹, Sakshi Kumari¹, Yamini Agrawal³, Preeti Verma¹, Aarti
Yadav^{1*} and Diwakar Kumar^{2*}**

¹Department of Microbiology, Shaheed Rajguru College of Applied Sciences for Women, University of Delhi, New Delhi, India

²Department of Microbiology, Assam University, Silchar, India

³Department of Botany, University of Delhi, Delhi, India

***Corresponding Author:** Department of Microbiology, Shaheed Rajguru College of Applied Sciences for Women, University of Delhi, New Delhi and Dr. Diwakar Kumar, Department of Microbiology, Assam University, Silchar, India.

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Abstract

Domain Archaea includes anaerobic prokaryotes like Methanogens which convert varieties of methyl compounds into methane by utilizing hydrogen to reduce acetate and carbon dioxide. *Methanobacteriales*, *Methanococcales*, *Methanopyrales*, *Methanomicrobiales* and *Methanosarcinales* are the five well-established orders classified by current taxonomy of methanogens. Not only are they found in extreme habitats, but they are also reported in various environments like the human body and mesophilic conditions. In humans, methanogens have been studied in the gastrointestinal tract, mouth, and vagina. Apart from the animal digestive tract, they are also found in the anoxic sedimented area and sewage sludge digestion. Methanoarchaea is an essential part of ammonia turnover and also considered a part of the human skin microbiome. The human body shelters a handful of methanogen species represented by *Methanobrevibacter oralis*, *Methanomassiliicoccus luminyensis*, *Methanobrevibacter smithii*, *Methanosphaera stadtmanae*, *Candidatus Methanomethylophilus alvus*, and *Candidatus Methanomassiliicoccus intestinalis*. Most methanogens are chemoautotrophs. They are a morphologically diverse group. Their application as probiotics in lactating cows, beef and cattle is of great significance. Methanogens affect climate vastly, and methane is one of the principal factors behind greenhouse fuel emissions. Biogas contributes to sustainable development by utilizing waste, and methane contributes to this renewable energy source. In this review, we examine the current knowledge about the Methanogens' possible beneficial or less favorable interaction

Keywords: Archaea; Methanogens; Sustainable Development; Methane; Biogas

Introduction

Methanogens are strict anaerobes that harvest carbon dioxide, hydrogen gas, formate, methanol, acetate, and other chemicals to methane or methane and carbon dioxide. It is by far the largest archaeal group comprising of various shapes and sizes classified into different classes based on phylogenetic analysis. They thrive in anoxic environments rich in organic matter: rumen and intestinal

system of animals, freshwater and marine sediments, etc. The cell walls of methanogens have a wide range of chemistries such as pseudomurin walls, methanochondroitin walls and the S-layer layer. As they utilise $H_2 + CO_2$, formate, methylated C_1 molecules, or acetate as energy and carbon sources for growth, methanogenic archaea have an uncommon sort of metabolism. Methane is the main by-product of their metabolism. Methane is a potential energy source; therefore, methanogens are of great ecological relevance.

Taxonomy

Methanogens are the methane-producing anaerobic archaea that belongs to the phylum Euryarchaeota and 5 orders i.e., methanococcales, methanobacteriales, methanopyrales,

methanosarcinales and methanomicrobiales (Figure 1). They are phylogenetically very diverse even though they share a set of similar physiological characteristics [1].

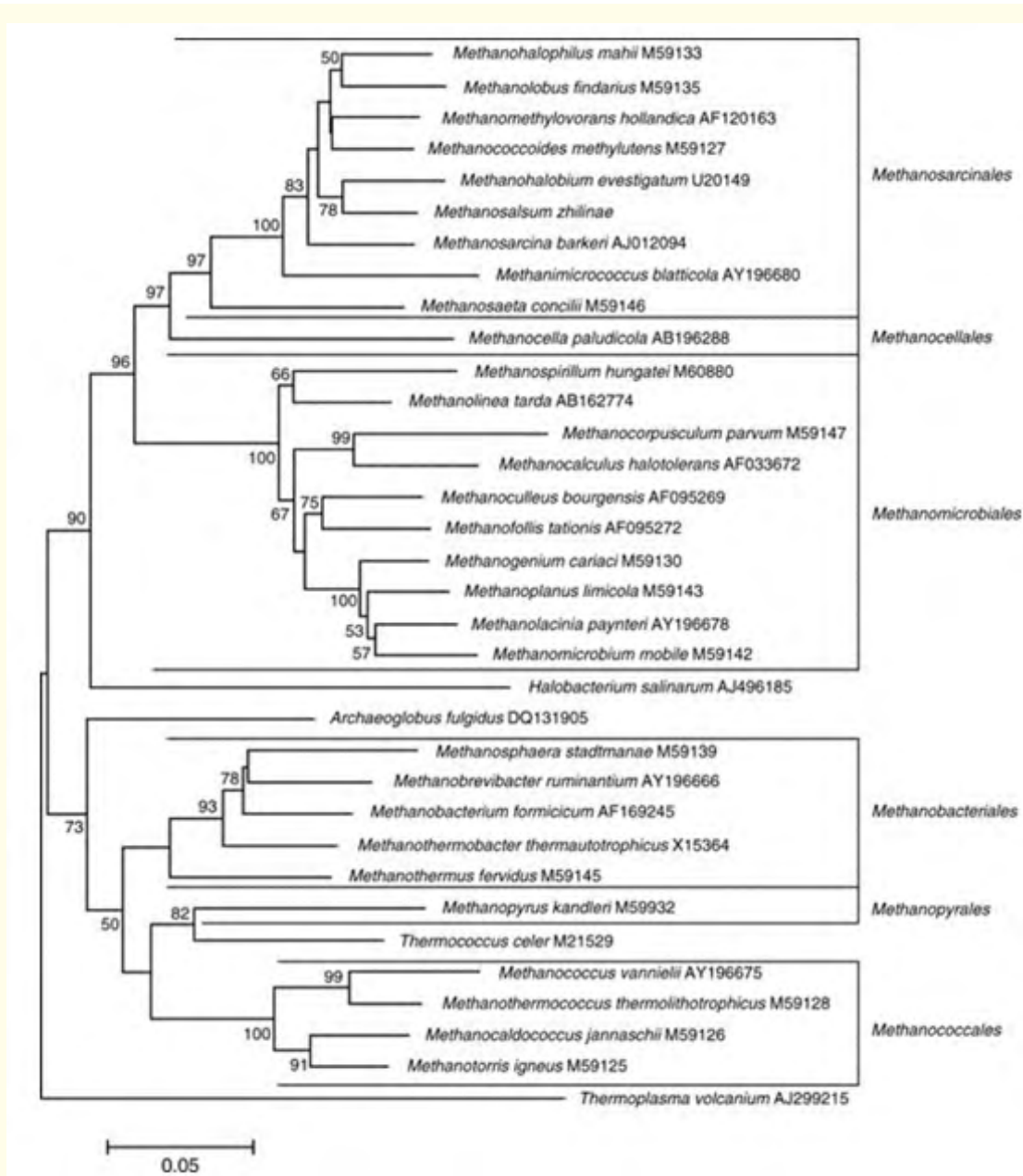


Figure 1: Phylogenetic tree depicting different classes of methanogens. (Taken from Liu, Y. (2010). Taxonomy of Methanogens. In: Timmis, K.N. (eds) Handbook of Hydrocarbon and Lipid Microbiology. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-77587-4_42).

16S rRNA gene sequences support this taxonomy because of the similarity between several physiological properties, e.g., substrates for methanogenesis, dietary requirements and morphologies. A marketing consultant of the clone lineage Rice Cluster I, suggests that the 16S rRNA gene sequence comparison of the stress SANAE, represents a novel order of methanogens, Methanocellales. Methanogens are found in a wide variety of anaerobic environments. By altering methanogenic substrates into methane, they catalyze the terminal step in the anaerobic food chain. Monophyletic origin of methanogens is not supported by the complexity of metabolic pathways; however, this is supported by phylogenetic evolution hypothesis based on DNA sequences [43].

Mode of nutrition

Methanogens are chemosynthetic autotrophic archaea [19].

Habitat

The most common habitat for methanogens includes anoxic sediments, such as those found in marshes, swamps, lakes, rice

fields, damp landfills, and animal digestive tracts, primarily those of ruminants that include cattle, sheep, and other species [33]. Methanogens associated with species of the genus *Methanobrevibacter* are found in herbivore guts [34].

Ruminants produce most of the methane among livestock as they harbour these methanogens in their rumen during feed digestion. Methanogens species are found in the cecum of horses and rabbits and other cecal animals, also in monogastric animal species' large intestines, such as humans and pigs, cellulolytic insects' hindguts, such as termites, etc., in hydrothermal vents and sewage sludge digesters. They are also found as endosymbionts of various anaerobic protozoa [33].

Morphology

Methanogens are a morphologically diverse group. They can be either spherical or rod-shaped. *Methanoplanus* genus occurs as thin plates with sharp edges [33]. Table 1 shows the diverse morphologies of the different genera of methanogens.

Order/Genus	Morphology	Substrates for Methanogenesis
Methanobacteriales <i>Methanobacterium</i> <i>Methanobrevibacter</i> <i>Methanosphaera</i> <i>Methanothermus</i> <i>Methanothermobacter</i>	Long rods Short rods Cocci Rods Rods	H ₂ +CO ₂ , Formate H ₂ +CO ₂ , Formate Methanol + H ₂ (both needed) H ₂ +CO ₂ H ₂ +CO ₂ , Formate
Methanococcales <i>Methanococcus</i> <i>Methanothermococcus</i> <i>Methanocaldococcus</i> <i>Methanotorris</i>	Irregular cocci Cocci Cocci Cocci	H ₂ +CO ₂ , Pyruvate + CO ₂ , Formate H ₂ +CO ₂ , Formate H ₂ +CO ₂ H ₂ +CO ₂
Methanomicrobiales <i>Methanomicrobium</i> <i>Methanogenium</i> <i>Methanospirillum</i> <i>Methanoplanus</i> <i>Methanocorpusculum</i> <i>Methanoculleus</i> <i>Methanofollis</i> <i>Methanolacinia</i> Methanosarcinales <i>Methanosarcina</i> <i>Methanobolus</i> <i>Methanohalobium</i> <i>Methanococcoides</i> <i>Methanohalophilus</i> <i>Methanosaeta</i> <i>Methanosalsum</i> <i>Methanimicrococcus</i>	Short rods Irregular cocci Spirilla Plate-shaped cells-occurring as thin plates with sharp edges Irregular cocci Irregular cocci Irregular cocci Irregular rods Large, irregular cocci in packets Irregular cocci in aggregates Irregular cocci Irregular cocci Irregular cocci Long rods of filaments Irregular cocci Irregular cocci	H ₂ +CO, Formate H ₂ +CO ₂ , Formate H ₂ +CO ₂ , Formate H ₂ +CO ₂ , Formate H ₂ +CO ₂ , Formate, Alcohols H ₂ +CO ₂ , Alcohols, Formate H ₂ +CO ₂ , Formate H ₂ +CO ₂ , Alcohols H ₂ +CO ₂ , Methanol, Methylamines, Acetate Methanol, Methylamines Methanol, Methylamines Methanol, Methylamines Methanol, Methylamines, Methyl Sulfides Acetate Methanol, Methylamines, Dimethylsulfide Methanol, Methylamines (H ₂ needed with any methanogenic substrate)
Methanopyrales <i>Methanopyrus</i>	Rods in chains	H ₂ +CO ₂

Table 1: Morphology and Substrates for Methanogenesis.

Genomics and molecular structure

Methanogens represent enormous phylogenetic diversity. Based on phylogenetic analysis, methanogens are divided into two major groups: class I and II. Class 1 methanogens are more similar to Methanomicrobiales physiologically rather than Methanosarcinales that grow on H_2/CO_2 or formate. Methane is produced by methanol, acetate, methylamines, and other C-1 compounds by the members of Methanosarcinales [1].

Ecophysiologicaly related WSA2 one of the potential candidate class of methanogen remains uncharacterized, despite its identification through 16S ribosomal RNA sequencing. It has been observed in a wide range of natural and engineered environments. [25]. In order to survive in soil and sediment conditions the genome of *Methanomassiliicoccus luminyensis* has several features, indicating its more extensive environmental distribution not only in the gastrointestinal tract. For *M. luminyensis*, its amino acid was encoded in the remnant ~17 kbp from the genome, which is not currently available, and no tRNA was detected [3]. *M. labreanum* and *M. marisnigri*'s genomes consist of one chromosome and no plasmids, which is also true for *M. hungatei* [1].

Genetic markers can be provided by all those methanogen genes that rescue auxotrophic mutation and those that determine resistance to agents active against methanogens. Non- methanogen markers must be expressed in the methanogen, and the same gene product must function there [17]. Methanogenesis evolves after the root is consistent with a recent analysis that places the archaeal root between the Different Putative Phylum-Level Lineages (DPANN) and the rest of Archaea [29].

Strains of methanogens

The various strains of methanogens include *Methanobacterium formicium*, *Methanobrevibacter arboriphilicus*, *Methanococcoides burtonii*, *Methanocalculus chunghsingensis*, *Methanococcus deltae*, *Methanobacterium wolfei*, and *Methanospirillum hungatei*. They mostly have one carbon, and the most versatile strain *Methanosarcina* uses seven substrates [43].

MT, MS, MM, MSP, and ZB (*Methanosaeta thermophila*, *Methanosarcina stordalenmerensis*, *Methanosarcina mazei*, *Methanosphaera stadtmanae* {hydrogenotrophic strain},)- were the five strains isolated from permanently and periodically

cold terrestrial habitats where ZB was closely related to *Methylomethalovorans*. Natural environment methanogens are psychrophiles or psychrotolerants. At a temperature of 5 - 28°C, *Methanosarcina* usually grows. Later on, from an anoxic hypolimnion of Ace Lake in Antarctica, two new species of methanogenic Archaea, *Methanococcoidesburtonii* and *Methanogeniumfrigidium*, were isolated. *Methanosarcinalacustris*, a psychrotolerant methanogen, was recently isolated from cold freshwater sediment.

The methanogens that were isolated exhibited different morphologies. MM, MS and MT had a morphology and cell arrangement similar to sarcina. They exhibited varying sizes. The MT cell aggregates were large with a 1mm diameter, and the cell morphology of ZB differed. They never formed aggregates and were motile irregular cocci. MSP strains were represented by motile irregular cocci of 0.3-0.6 micron meter diameter. According to 16S rRNA data, strain MHu and MS were identical, having 98.5% similarity with lacustris. ZB was 99.9% similar to *Methyllovoranshollandica*, and MSP was classified as *Methanocorpusculum* [31].

Methanogenesis

Methanogenesis, or bio-methanation, is a biological process in which microbes called methanogens produce methane. Methane-producing organisms have only been found in the domain Archaea, which is genotypically separate from both eukaryotes and bacteria, albeit many lives close to anaerobic bacteria.

Methanogens can utilize as wide variety of substrates for methanogenesis. Glucose and other carbohydrates can be transformed to CH_4 , but only in reactions involving methanogens and other anaerobes. Almost any organic substance, including hydrocarbons, can be transformed into $CH_4 + CO_2$ with the correct combination of organisms [33].

The CO_2 -type substrate contains CO_2 , which can be converted to CH_4 by using H_2 as an electron donor. Methanol (CH_3OH) is one of the various methylated substrates. As an alternative to H_2 , methanol can be reduced by oxidizing some CH_3OH molecules to CO_2 to create the electrons needed to reduce other CH_3OH molecules to CH_4 [33]. The cleavage of acetate to $CO_2 + CH_4$ is the final step in the methanogenic process (Table 2). When all other electron acceptors

have been exhausted, methanogenesis is the metabolism that degrades carbon. Due to the lack of oxidants in waterlogged soils, methanogenesis is a prominent process for decomposing organic matter in many wetlands, despite its abysmal energy production [30].

Methane can be created in flooded sediments by two distinct processes carried out by methanogens. Methanogens can utilize acetate to create methane in a process called acetoclastic methanogenesis or acetate fermentation when organic matter

fermentation produces organic acids over the availability of alternate electron donors. Compared to other anaerobic metabolic pathways, the energy yield of acetoclastic methanogenesis is relatively low. Only two genera of methanogens perform acetoclastic methanogenesis: *Methanosarcina* and *Methanosaeta*. In the absence of acetate, methanogens can undergo hydrogen fermentation with CO₂ reduction, in which hydrogen acts as a source of electrons and energy, and CO₂ acts as a source of carbon and an electron acceptor [30].

Reaction		$-\Delta G' \text{ (KJ) mol}^{-1}$	Organisms Catalyzing These Reactions
Acetate splitting 1. CH ₃ COOH	$\rightarrow \text{CH}_4 + \text{CO}_2$	28	Some methanogens (<i>M. barkeri</i> , <i>M. mazei</i> , <i>M. sohngeni</i>)
CO ₂ Reduction 2. CO ₂ + 4H ₂	$\rightarrow \text{CH}_4 + \text{H}_2\text{O}$	17.4	Most methane bacteria

Table 2: Showing the chemical reactions and associated free energy yield for methane-producing pathways of acetate splitting and CO₂ reduction.

Methane production in ruminants

Some of the significant methanogens are *Methanomicrobium mobile* and *Methanobrevibacter ruminantium*. Clones identified by Wright and his colleagues from bovine rumen fluid were similar to the cultivation of methanogens of the order Methanobacteriales. Using the temporal gradient gel electrophoresis, scientist Nicholson examined sheep and cattle rumen. Most clones belong to the largest group of clones, i.e., Wright and his colleagues gave the genus *Methanobrevibacter*, and this conclusion is from Venezuela through analysis.

Methanobrevibacter ruminantium belonged to methanogens' largest group and was detected by Whitford and his co-workers in cattle and dairy fields. A clone library was made from cattle rumen fluid, based on the study by Prince Edward Island, which fed a diet of potato by-products to cattle. Moreover, based on geographical location, methanogen clones are unique [28].

They play an essential role in the digestive system of ruminants. Most of the anaerobic microbes facilitate the breakdown of cellulose which occupies the rumen and initiate the fermentation process

[28]. Methane can be produced in the lower gastrointestinal tract or rumen; in most cases, it is exhaled through the mouth and nose.

Experiments have been conducted to study the relationship between methanogens and other microorganisms and the population of methanogens in the rumen of cattle and sheep. Depending on the diet and geographical location of the host, the rumen methanogen species differ in methanogenesis, which is reduced by supplementation of monensin, organic acids, and lipids, modifying dietary composition or plant compounds within the diet. The population of methanogens in the rumen is affected directly or indirectly in the mitigation method and results in varying degrees of efficacy. Carbohydrate fermentation results in hydrogen production, and its end product can inhibit the metabolism of the rumen. During ruminal digestion, the gross energy consumed is around 2% to 12% converted to enteric methane and contributes around 6% of global anthropogenic greenhouse gas emissions [2].

86 million metric tonnes (Tg) of methane per year is produced by domesticated ruminants such as sheep, goats, and cattle. Approximately 55.9 Tg are from beef cattle, 18.9 Tg are from

dairy cattle, and 9.5 Tg are from goats and sheep. According to estimated data, the global yearly methane contribution of buffalo is around 6.2–8.1 Tg, 0.9–1.1 Tg from camels, and approximately 0.9–1.0 Tg and 1.7 Tg methane production within the hindgut of pigs and horses, respectively [13]. Therefore, ruminants play an essential role in food production and employment, as well as global acceptance of sustainable ruminant production through probiotics supplementation.

Defaunation treatment

It is the process of removing protozoa from the rumen, used to investigate its role and study the effect of methane production. Methanogens share a symbiotic relationship with rumen protozoa. More population of rumen protozoa may decrease methane production within the rumen. This treatment uses copper sulfate acids, triazine, lipids, surface-active chemicals, ionophores, tannins, and saponins. Studies have proven that viable protozoa transfer to defaunated animals does not occur readily through contact with feed or feces of animals nor with direct contact [13].

Biogas generation

Methanogens may contribute to resolve future energy problems and act as a storable energy carrier. By the anaerobic breakdown of methanogenic bacteria, biogas is produced, a methane-rich fuel [7]. The greenhouse gas emission resulting from fossil energy sources have been reduced during the last decade due to biogas production from organic materials. Biogas formation primarily depends upon the activity of microbial organisms involved in biogas formation-methanogens [24].

A study was conducted by Mulat and their team, which aimed to investigate flexible biogas production by changing feeding intervals and its effect on the process of performance. This was done by terminal restriction fragment length polymorphism (T-RFLP) analysis of 16S rRNA and *mcrA* genes. The quantification of hydrogenotrophic methanogenesis (HM) and acetoclastic methanogenesis (AM) was done by analyzing carbon isotope signatures of methane and carbon dioxide. By molecular methods, the correlation between methanogenesis pathways was studied by isotope analysis, and methanogenic community structures were further assessed [23].

An up-and-coming and low-cost technology for managing renewable power intermittency and over-generation, Power-to-Gas (P2G) is the process for converting electricity to chemical energy in the form of hydrogen (H_2) via electrolysis. As one of the renewable energy carriers, the biogas obtained via anaerobic digestion (AD) has gained worldwide interest. Specific bacterial groups decompose organic polymer into acetate, H_2 , and CO_2 , which are further converted into CH_4 by methanogens in an AD process [42].

The production of biogas can be affected by parameters such as pH, organic acid concentration, loading rate, and others. The more depleted delta values after feeding were hypothesized to indicate a shift towards the predominance of hydrogenotrophic methanogenesis. Since hydrogenotrophic methanogenesis is associated with a more significant fractionation effect than acetoclastic methanogenesis, these pathway changes would result in the depletion of isotope composition. During acetoclastic methanogenesis, *Methanosaeta* spp. is weaker than *Methanosarcina* in isotope fractionation [21].

Using operational taxonomic units and amplified rRNA gene restriction analysis (ARDRA), all biogas plants were assigned to orders *Methanomicrobiales* and *Methanosarcinales* belonging to the phylum Euryarchaeota. The members of the genus *Methanoculleus* were found to be predominant by ARDRA. In full-scale biogas reactors, the influence of syntrophic relationships within microbial communities on carbon fluxes is required [24]. During temperature adaptation, a next generation sequencing -based metagenomic approach was applied to follow the development of microbial community structure in biogas reactors. As indicated by strongly increased VFA levels, the temperature changes temporarily destabilized the AD system. The higher sensitivity of methanogenic Archaea causes this destabilization. The methanogenic archaeal community suffered transformation in response to temperature elevation in line with the bacterial reorganization. The most abundant genus found was *Methanosaeta*, while *Methanosarcina*, *Methanospirillum*, and *Methanothermobacter* genera were detected moderately under mesophilic operation [26].

Higher biogas production was led by less frequent feeding of a higher amount of substrate at once while keeping the same

overall organic load rate. Methanogenic communities remained stable, whereas bacterial community composition was influenced by the changes in the feeding intervals [23]. Overall metabolic activity changes of microorganisms and substrate availability also contributed to the fluctuation of this isotope composition of methane. The short-term activity changes of methanogens in biogas reactors can be detected faster through stable isotope fingerprinting. The overall gas yield not only changes by the alteration of substrate feeding but makes the electrical production of biogas plants more flexible; hence it could be a good strategy. To optimize the demand driven biogas production, more research with different intervals is needed [21].

Current applications of probiotics in calves

Calves have specific functionalities based on the types of oligosaccharides they have. Recent studies show that deeding fructo-oligosaccharides in combination with bovine spray serum reduced the severity of enteric disease in calves. Additions of galactosyl-lactose to milk replacer have a more beneficial effect on the growth of dairy productivity. Minimal benefits of probiotics are likely to happen in healthy calves. An *in vivo* study shows that cello-oligosaccharide (CE) feeding improved dairy feed efficiency in calves. This results in more ruminal fermentation and an increase in total short-chain fatty acids [37].

Effect of supplementation with probiotics/prebiotics on the performance of heifers, lactating cows, beef, and cattle

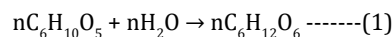
Rumen microorganisms increase fibre digestion, and selectively probiotics have positive effects on the synthesis of microbial proteins and in the process of cellulolytic. In dairy farms, at a more concentrated level, mainly probiotics that increase lactic acid production belong to lactate-producing bacteria and yeast. With the addition of yeast in both lactating and growing animals, diets gradually improved their productivity. Rumen fermentation rates and their patterns are affected by the mode of action of yeast.

Active dry yeast strains improved the population of ciliate protozoa by stimulating pH. In the growth of more fiber-degrading activity, less acidic ruminal shows more benefits than acidic ruminal, which also increases cellulosic microorganisms [37].

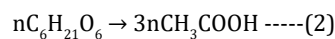
Biorefineries of food waste

Bacteria are involved in the methanogenic phase of anaerobic decomposition from a single group that includes several species with varying shapes and cell structures. There are two types of strictly anaerobic microorganisms: those that consume hydrogen and those that degrade acetic acid (acetoclastic methanogenic bacteria) (hydrogenotrophic methanogens). The first route is the most important for methane formation, accounting for roughly 70% of total methane production. Acetoclastic methanogenic bacteria produce methane from acetate. They grow slowly (minimum doubling time of 2–3 days) and are unaffected by the hydrogen concentration in the biogas. Hydrogen-consuming methanogenic bacteria produce methane from hydrogen and CO₂. This reaction serves a dual purpose in the AD process, producing methane while removing gaseous hydrogen. [27]

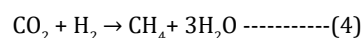
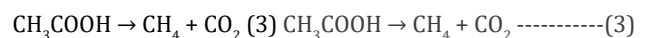
Macromolecules are broken down into smaller molecules in hydrolysis. Fermentative bacteria that produces Exo-enzymes are catalyzed by it, as illustrated in Equation 1. *Clostridium thermocellum* and *Bacteroides succinogenes* are two examples of these bacteria.



Acidogenesis is the second stage, in which acidogenic bacteria (*Clostridium butyricum*) convert smaller molecules into volatile fatty acids such propionic, acetic, and butyric acid, as well as various by-product gasses like ammonia, carbon dioxide, hydrogen sulfide, alcohols, and aldehydes, as illustrated in Equation (2).



The conversion of acetic acid into acetate, results in formation of carbon dioxide and methane (Equation 3) by acetoclastic methanogens such as *Methanosarcina* and *Methanosaeta*, is the third and fourth steps, respectively. By using CO₂ as a carbon source and hydrogen as a reducing agent hydrogenotrophic methanogens generate CH₄ (Equation 4).



AD or anaerobic fermentation yield products such as methane (CH₄), volatile fatty acids (VFAs), such as propionic acid, butyric

acid, acetic acid, iso-butyric acid, and hydrogen [35]. When compared to a single-stage system, a two-stage anaerobic system that combines hydrogen or ethanol with methane fermentation has the potential to improve the substrate's energy recovery efficiency and is regarded as a promising technology. Trace elements such as cobalt (Co), nickel (Ni), zinc (Zn), manganese (Mn), and iron (Fe) are essential in the methanogenesis step of AD, which catalyzes key metabolic steps by action of acetyl CoA synthase and methyl coenzyme M reductase and require sufficient amounts of Fe, Ni, and Co. Low CH₄ production results from the inefficiency of the methanogenesis step. Methanogenesis is also inhibited by AD metabolic by-products such as ammonia (NH₃) [40].

Using the biorefinery concept, numerous products such as protein, animal feed, enzymes, organic acids, flavors and colorants, bio-fertilizers, bioplastics, and biofuels can be produced simultaneously and sequentially from food waste [35]. Furthermore, the biorefinery would increase the commercial value of AD of food waste through separated treatment based on substrate components [28].

Recovery of energy from food waste

Food waste is a future sustainable energy source due to its nutrient-rich nature. A two-stage anaerobic system that combines hydrogen or ethanol with methane fermentation has the potential to improve the substrate's energy recovery efficiency. It is observed that the energy carried by H₂ during acidogenesis can account for up to 30% of the total energy recovered [6].

There are three potential solutions to this problem: (1) reduce H₂ production in the acidogenic reactor; (2) use H₂ while simultaneously reducing CO₂ to acetate by homoacetogens in a coupling system and (3) use H₂ and CO₂ in the methanogenic reactor via hydrogenotrophic methanogenesis. The addition of H₂ to the methanogenic reactor improved methane recovery. Because of the highly thermodynamically favorable nature of hydrogenotrophic methanogenesis, using H₂ in a methanogenic reactor would be preferable.

The diversion of acidogenic off-gas from an acidogenic leach bed reactor (LBR) to a methanogenic up-flow anaerobic sludge blanket (UASB) for utilization of H₂ and CO₂ via direct hydrogenotrophic methanogenesis was investigated in this study as a strategy

for improving overall energy recovery from two phases AD of food waste. Adding H₂ to a methanogenic reactor could increase methane gas generation and improve the CH₄ content of the mixed biogas [20]. The general performance of the two-phase AD system, a shift of acidogenic metabolic pathway, and overall CH₄ recovery under the configuration of acidogenic off-gas diversion to the methanogenic reactor were investigated to assess the feasibility and efficiency of this strategy [41].

Biohydrogen generation

Increasing greenhouse pollution, the inability to replenish the depleting fossil fuels, the ever-increasing gap between energy requirements, and the energy crisis stimulate the need for alternative sustainable and eco-friendly fuels worldwide. The H₂ production, among biological H₂ production processes, has been considered a viable and effective method. This occurs at ambient pressure, is less energy-intensive, and is more environmentally friendly. As a renewable byproduct, integrating the acidogenic H₂ production process with an anaerobic methanogenic process enhances substrate degradation efficiency along with H₂ and CH₄ generation [38]. Acetate, a favourite substrate for methanogenesis, converts it into methane and carbon dioxide. In order to inhibit methanogens, Microbial Electrolysis Cell from the reactor is exposed to the air. Because of the fermentative nature of methanogens, they compete with exoelectrogens for the substrate, which is quite severe with a complex substrate, such as glucose and wastewater. It appears that there will be a significant decrease in energy loss if methane production can be replaced by exoelectrogenic and hence shows that in the MEC system, suppression of methanogens leads to enhancement of overall hydrogen recovery [4].

During the butyrate-type fermentation process, when the hydraulic retention time of the reactor was long enough for the growth of methanogens, it did not thoroughly inhibit the methanogenic activity when pH was 4.5. Biogas production was increased by 27% with the addition of nitrate to the reactor. The increased biogas portion was attributed to hydrogen gas by inhibiting methanogenic activity [16]. Since the acidogenic reactor for hydrogen production would be the first unit in a wastewater treatment plant and the effluent would be a second stage, which could be a methanogenic anaerobic reactor, the low efficiencies of organic matter removal do not represent an environmental

problem [8]. Coenzymes such as anofuran, methanoprotien, and coenzyme M, employed by methanogens, play an essential role as C1 carriers in the Biohydrogen genesis. In methane biosynthesis, Coenzyme M is a cofactor involved in the terminal step and acts as a C1 carrier in methanogenesis [4].

From the acidogenic process as primary substrate and the process of integration facilitated utilization of residual carbon source along with generated volatile fatty acids in the methanogenic process involves methane generation associated with additional substrate degradation. For sustainable H_2 generation with wastewater as substrate, the integration process appears to be the most promising approach [38]. Many technological changes are still being faced by MECs, such as membrane-associated pH imbalance and methane production on the cathode. However, reactor design and consistent advancements in technology have made it promising for Biohydrogen production [4].

Greenhouse fuel emissions

Greenhouse gasses like CO_2 , Methane, Nitrous oxide, and ozone are foremost responsible for global warming all over the world and result in the upliftment of temperature. Global warming is caused by infrared radiation due to methane. CH_4 is the principal factor of herbal gasoline and full-of-life greenhouse gasoline (GHG). Upon escaping into the atmosphere, greenhouse gasses, absorbs power and slows the price at which warmness leaves the planet. In the case of methane, this strength is absorbed remarkably well. This is a naturally occurring process which is referred to as the greenhouse effect, and besides that the temperature of earth would fall freezing. However, with the increase in greenhouse gasoline emissions over the remaining few centuries, the greenhouse impact has grown persistently at a rate which is alarming [32].

Earth's 2nd most plentiful greenhouse fuel after carbon dioxide is methane but it is comparatively short-lived. This comparison jumps to approximately 80 times over these 20 years that for the last 100 years methane has been considered 28% more omnipotent than carbon dioxide. As methane is released into the air, it reacts in various damaging ways. For one, methane leaves the ecosystem through oxidation, forming water vapor and carbon dioxide. So, methane does not solely contribute to world warming at once but also, in a roundabout way, via the launch of carbon dioxide.

Additionally, methane reacts with hydroxyl radicals (OH) at some point in the oxidation process. These naturally happening molecules act as a "detergent", cleansing methane and much different pollution from the air. Air pollutants can be eliminated by reducing the number of hydroxyl radicals with the help of methane. Trace gas like methane had a total concentration of 1.8 ppb in 1774 and increased by 5.7 ± 1.2 parts per billion (ppb) every year between 2007 and 2013 [13].

Methane is more potent as compared to carbon dioxide in causing global warming. Additionally contributes to the formation of the ozone, decreasing air quality, and untimely human deaths, primary to several fitness problems in animals and lowered crop yields. The agriculture sector is responsible for 50-60% of methane emissions, especially livestock production. Ultimately, reducing these effects can be done by lowering the quantity of methane in the surroundings. By focusing on anthropogenic or artificial, methane emissions alone, we can decrease the rate of world warming considerably [18].

Effects on climate

Methanogenesis can potentially be a hazard to the environment. Methane is a greenhouse gas because it absorbs infrared radiation. Methane levels in the atmosphere have been increasing for the past 200 years. Methanogenesis in cattle and the decay of natural cloth is a significant contributor to world warming. It may no longer be a leading contributor as it works on natural fabric, that is being using up atmospheric carbon dioxide when it was created. Methanogenesis can also be exploited to deal with natural waste and produce beneficial compounds. It can moreover be beneficially exploited to deal with herbal waste, to produce recommended compounds, and the methane can be gathered and used as biogas, a fuel. It is a natural count disposed of through landfill is damaged down and is the principal pathway [9,14].

Role of methanogens in methyl-mercury toxicity

Mercury contamination of food occurs mostly from industrial releases into the water, where it is converted to methylmercury by Methanogenic bacteria. As marine species absorb methyl mercury, it enters the food chain and is eventually swallowed by people. By using elemental mercury as the sole source of mercury,

anaerobic bacteria produce toxic methylmercury. Researchers in one study collected a sample from the San Jacinto River estuary (Texas, USA) to investigate the production of methylmercury. The methylmercury synthesis was significantly reduced after adding a methanogen inhibitor enzyme. The findings revealed the importance of methanogens in producing methylmercury [39].

In one study, the synthesis of methylmercury was investigated using nine methanogens with the *hgcAB+* gene-*Methanocella paludicola* SANA, *Methanofollis liminatans* GKZPZ, *Methanocorpusculum bavaricum*, *Methanosphaerula palustris* E1-9c, and *Methanococcoides methylutens*. Eight out of nine methanogens were discovered capable of generating methylmercury at rates comparable to those in recognized mercury-methylating bacteria [11]. Another study showed that inhibitors of methanogens and sulfate-reducing bacteria significantly reduced methylmercury production [5].

Methylmercury can be eaten through food by persons whose jobs are not directly tied to mercury exposure, which can have negative health consequences. Therefore, methylmercury is a harmful element that demands the attention of environmental health professionals. Inorganic mercury makes up the majority of mercury in the air, but organic mercury makes up the majority in human bodies. Fish and shellfish consumption accounts for 80 percent to 90 percent of organic mercury in the human body, with methylmercury accounting for 75 percent to 90 percent of organic mercury in fish and shellfish. Fish and shellfish consumption for more than 30 days was responsible for 75% of blood mercury [22].

Methylmercury is exceptionally hazardous, and its toxicity varies depending on its type, intake route, exposure level, and individual susceptibility. Even if the mother shows no signs of poisoning, methylmercury exposure during pregnancy can raise the chance of silent childbirth and the birth of kids with abnormalities or severe nervous system problems [10,36].

In the 1950s, incidents of methylmercury poisoning in Minamata, Japan, showed that methylmercury poisoning had devastating consequences on fetal brains. Babies suffered central nervous system disorders such as paralysis and IQ disorders, but moms showed little or no signs of poisoning [12]. Mercury can enter the body through air, food, drinks, and teeth treated with amalgam.

Of the mercury that enters the human body, methylmercury is the most toxic to the human body and has a high proportion of human residue, around 95% [15].

The total mercury in the air is estimated to be 10 ng/m³, with monomethyl mercury and dimethylmercury accounting for 22% of the total. However, the air is not a significant cause of methylmercury exposure, as the average amount of methylmercury inhaled by adults is only 0.04 g/d [12].

On the other hand, mercury that has penetrated the sea, rivers, and streams binds to various biomolecules and lives in a stable form. Methanogens and other mercury-methylating must methylate inorganic mercury before methylmercury can be produced. When methylmercury is produced, it rapidly disperses to marine organisms and is then condensed within them. Methylmercury accumulates in humans who consume these marine species causing biomagnification of methylmercury. Thus, we can say that methanogens play a significant role in methyl-mercury toxicity, which causes lethal effects in humans.

Conclusion

Methanogens play a pivotal role in maintenance of the ecological processes and may assist in resolving major global concerns such as waste management, by breaking down the organic material that would have polluted the water bodies and may have led to environmental degradation, methanogens do their best to protect the environment. and also play a significant role in ammonia turnover. Methanogens are hence of enormous significance; they have implied in all the biofuels generations which contribute less towards environmental degradation and benefit humanity. However, at the same time methanogenesis may also contribute to global warming unless methane is harnessed into other processes and further research needs to be taken for this sector.

Availability of Data

All data generated or analyzed during this study are included in this published article.

Ethics Approval and Consent to Participate

The study does not contain any studies with human participants or animals that require specific ethical approval. All the data has been collected from secondary sources.

Consent for Publication

All the authors give their consent for publication.

Competing Interests

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

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Conflict of Interest

The authors declare that no conflict of interest exists.

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Author Contribution Statements

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