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Biomethane: The energy storage, platform chemical and greenhouse gas mitigation target

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ABSTRACT

Results in three areas of anaerobic microbiology in which methane formation and utilization plays central part are reviewed. a.) Bio-methane formation by reduction of carbon dioxide in the power-to-gas process and the various possibilities of improvement of the process is a very intensively studied topic recently. From the numerous potential methods of exploiting methane of biological origin two aspects are discussed in detail. b.) Methane can serve as a platform chemical in various chemical and biochemical synthetic processes. Particular emphasis is put on the biochemical conversion pathways involving methanotrophs and their methane monooxygenase-catalyzed reactions leading to various small molecules and polymeric materials such as extracellular polysaccharides, polyhydroxyalkanoates and proteins. c.) The third area covered concerns methane-consuming reactions and methane emission mitigation. These investigations comprise the anaerobic microbiology of ruminants and approaches to diminishing methane emissions from ruminant animals.

1. Bio-methane: an energy storage medium

Because of the increasing levels of greenhouse gas emissions and the rising global energy demand new technologies for the generation of environmentally friendly energy carriers are needed. Renewable energy sources like solar and wind energy have great potential, but their utilization is difficult due to their fluctuating production and consumption. In large electricity networks, renewable power sources with a low input can be balanced by conventional power generation, but a higher percentage of renewables would need improved energy storage. Whereas batteries, compressed air, flywheels or capacitors are suitable but expensive tools for the short-term storage of electricity, long-term storage could be realized with hydrogen as an energy carrier. Up to now, problems with fluctuating and intermittent electricity from renewable power sources have only occurred in local power grids with a high percentage of renewables. In the future, high input of renewable electricity is expected to be fed into larger power grids too. With power-to-gas technology, electricity is converted into hydrogen by water electrolysis. The hydrogen can be stored in pressure tanks and when needed can be reconverted into electricity with fuel cells or hydrogen combustion engines [1]. Besides its use as an energy carrier for electricity, mobility and heat, hydrogen can be utilized as a raw material for the chemical industry or for the synthesis of various hydrocarbon fuels such as methane. Fig. 1 shows the main components of a power-to-gas system and the various types of applications for it.

The conversion of carbon dioxide to a useful carbonic compound may contribute to carbon dioxide reduction in the atmosphere. However, carbon dioxide is a thermodynamically stable compound and its reduction requires high energy and electroreductive processes. Various carbon dioxide reduction methods using chemical

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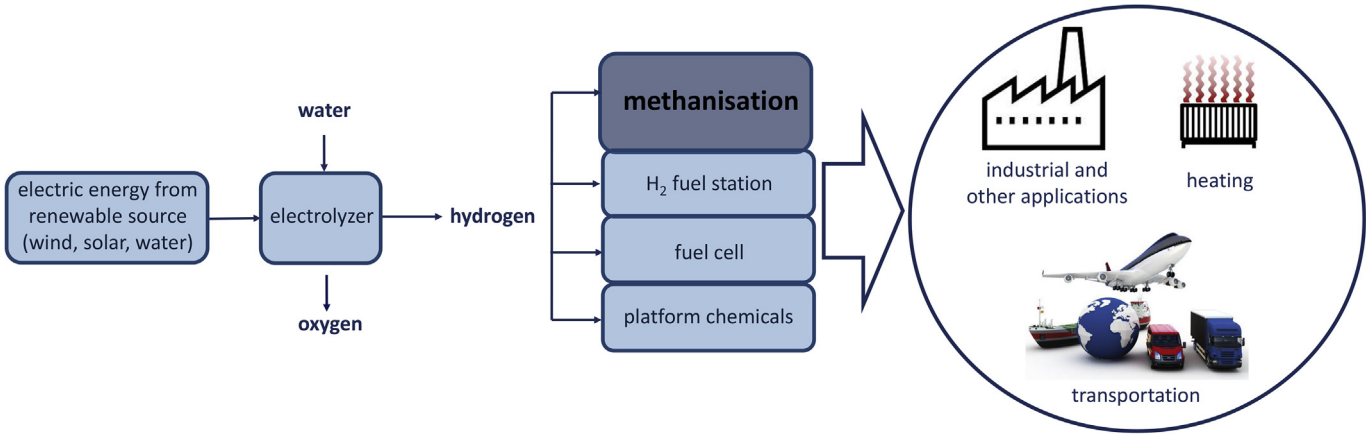

Fig. 1. Scheme of the power-to-gas concept.

and biological reactions have been proposed and investigated, for example catalytic hydrogenation, electrolytic reduction and photosynthesis by algae. The potential of catalysts for carbon dioxide conversion has been discussed in recent reviews [2] [3]. In the presence of noble metals such as Rh, Ru and Pd carbon dioxide can be reduced with reducing agents including hydrogen or electron-rich chemicals. However, the inert property of carbon dioxide, together with lower reactivity in various reactions, needs energy-intensive operating conditions including high temperature (300–600 °C) and high pressure (higher than 10 atm).

Biological conversion of carbon dioxide requires only mild operation conditions. Biological fixation using the photosynthetic function of the microalgae *Chlorella* and *Synechocystis* sp. can save energy compared with catalytic conversion requiring energy-intensive reaction conditions. The reaction conditions for photosynthetic fixation are mild, requiring about 20–30 °C and 1 atm. Light has to be provided for photosynthetic reactions, making reactor design difficult and the microalgae system requires land, water and climate resources that are seldom found near a carbon dioxide-generating plant. Instead of using the photosynthetic function of microalgae, methanogens, which are known to have the capability to utilize carbon dioxide as an electron acceptor, are proposed [4]. Methanogens have been classified as methylotrophic, acetoclastic and hydrogenotrophic. Methylotrophic methanogens are known to use methyl compounds such as methanol, dimethyl sulfide, trihalomethanes, chloromethanes, etc. Acetoclastic methanogens produce methane from acetate that is a major intermediate produced from anaerobic digestion of organic matter. Hydrogenotrophic methanogens are known to use carbon dioxide as an electron acceptor and hydrogen as an electron donor. A recent study reported that hydrogenotrophic methanogens were enriched in an electrochemical bioreactor [5]. Hydrogenotrophic methanogens can produce bio-methane from a hydrogen–carbon dioxide mixture without other organic carbon sources. We propose to call the product of this reaction bio-methane to distinguish it from “biomethane”, a term commonly used for upgraded biogas.

The importance of syntrophic relationships among microorganisms participating in biogas formation has been emphasized, and the regulatory role of in situ hydrogen production has been recognized [6]. It was assumed that the availability of hydrogen may be a limiting factor for hydrogenotrophic methanogens. This hypothesis was tested under laboratory and field conditions by adding a mesophilic (*Enterobacter cloacae*) or thermophilic (*Caldicellulosyruptor saccharolyticus*) hydrogen-producing strain to natural biogas-producing consortia. The substrates were wastewater sludge, dried plant biomass from Jerusalem artichoke and pig manure. In all cases, a significant intensification of biogas production was observed.

Hydrogenotrophic methanogens had been considered as key species for the anaerobic digestion of industrial wastewater sludge and municipal organic waste. In a study, a volumetric device and a test procedure were developed for measuring the specific hydrogen utilization rate (HUR) of anaerobic sludge [7]. Results showed that HUR values were highly influenced by sludge concentrations because of limitation on hydrogen mass transfer. A field survey confirmed that HUR exhibited a good relationship with specific methanogenic activity (SMA) and reactor performance. An anaerobic system with a relatively high HUR was found to be beneficial for maintaining hydrogen partial pressure at an appropriately low level. Moreover, such a system was thermodynamically favorable for the syntrophic degradation of volatile fatty acids.

The conversion efficiency of hydrogen by methanogens was also examined [8]. Hydrogenotrophic methanogens were enriched in a fixed bed reactor by feeding a gas mixture of carbon dioxide and hydrogen. According to the stoichiometry, 4 mol of hydrogen is needed to reduce 1 mol of carbon dioxide. In order to obtain engineering data for reactor design, the gas retention time in the fixed bed reactor was changed by varying the flow rate of a gas mixture of carbon dioxide and hydrogen. Conversion of carbon dioxide to bio-methane by hydrogenotrophic methanogens occurred according to the stoichiometry of the chemical equation. The carbon dioxide conversion rate was 100% when the gas retention time was 3.8 h or longer.

A novel method to convert carbon dioxide to biogas with a high content of methane in an anaerobic system with a lab-scale upflow anaerobic sludge blanket reactor at 35 °C was developed [9]. In a series of experiments, the reactor was run with and without carbon dioxide-saturated solutions including volatile fatty acids (VFAs) as sources of reductant. The concentration of dissolved carbon dioxide in the influent solutions was 2.2–6.1 g/L, with corresponding chemical oxygen demand (COD) values of 2.6–8.4 g/L. Overall carbon dioxide removal values of 2.7–20 g/day (49–88% conversion) were obtained for the organic loading rates (OLR) and carbon dioxide loading rates of 8–36 g COD/L/day and 6–26 g CO_2/L/day, respectively, with methane purity above 70%. Also, VFA and COD removal was in the range of 79–95% and 75–90%, respectively. Methanogenic activities of the cultures with the concentrations measured as volatile suspended solids (VSSs) were 0.12–0.40 L CH_4/g VSS/day with the highest value for the system containing acetic acid.

For examination of hydrogen conversion various reactor systems were developed [10]. An innovative setup has been

assembled, which comprised two-stage reactors to achieve biogas upgrading coupled to carbon dioxide reduction with external hydrogen and subsequent conversion into bio-methane by hydrogenotrophic methanogenesis. In this configuration, the biogas produced in the first reactor was transferred to the second one, into which hydrogen was injected. This arrangement was tested at both mesophilic and thermophilic conditions. After hydrogen addition, the produced biogas was upgraded to an average methane content of 89% in the mesophilic reactor and 85% in the thermophilic one. Under thermophilic conditions, a higher efficiency of methane production and carbon dioxide conversion was recorded. The relative abundance of the archaeal community markedly increased upon hydrogen addition with *Methanoculleus* as the dominant genus. Moreover, *Thermoanaerobacteraceae* were likely involved in syntrophic acetate oxidation with hydrogenotrophic methanogens in the absence of acetoclastic methanogenesis.

The procedure was examined under different conditions, at mesophilic and at thermophilic temperatures. Both mesophilic and thermophilic anaerobic cultures were enriched to convert carbon dioxide to methane by addition of hydrogen [11]. Enrichment at a thermophilic temperature (55 °C) resulted in a carbon dioxide and hydrogen bioconversion rate of 320 mL CH_4/g VSS/h, which was more than 60% higher than that under a mesophilic temperature (37 °C). Different dominant species were found in mesophilic- and thermophilic-enriched cultures, as revealed by PCR–DGGE. Nonetheless, they all belonged to the order *Methanobacteriales*, which can mediate hydrogenotrophic methanogenesis.

Biogas upgrading was then tested in a thermophilic anaerobic reactor under various operation conditions. By continuous addition of hydrogen to the biogas reactor, a high degree of biogas upgrading was achieved. The produced biogas had a methane content around 95% at steady state at a gas (mixture of biogas and hydrogen) injection rate of 6 L/L/day. The increase of gas injection rate to 12 L/L/day resulted in a decrease of methane content to around 90%. Further study showed improvement of gas–liquid mass transfer up to a methane content of around 95% by increasing the stirring speed. Finally, a methane content around 90% was achieved with the gas injection rate as high as 24 L/L/day.

The possibility of converting hydrogen to methane and simultaneous upgrading of biogas was investigated in both batch tests and a fully mixed biogas reactor, simultaneously fed with manure and hydrogen. Batch experiments showed that hydrogen could be converted to methane by hydrogenotrophic methanogenesis with conversion of more than 90% of the consumed hydrogen to methane [12]. The hydrogen consumption rates were affected by both hydrogen partial pressure and mixing intensity. Inhibition of propionate and butyrate degradation by hydrogen (1 atm) was only observed under high mixing intensity (shaking speed 300 rpm). Continuous addition of hydrogen (flow rate of 28.6 mL/L/h) to an anaerobic reactor fed with manure showed that more than 80% of the hydrogen was utilized. The propionate and butyrate level in the reactor was not significantly affected by hydrogen addition. The methane production rate of the reactor with hydrogen addition was 22% higher, compared to the control reactor only fed with manure. The carbon dioxide content in the produced biogas was only 15%, while it was 38% in the control reactor. However, the addition of hydrogen resulted in an increase of pH (from 8.0 to 8.3) due to the consumption of bicarbonate, which subsequently caused a slight inhibition of methanogenesis.

Most of the studies indicated that a major bottleneck in the power-to-gas process is the gas–liquid mass transfer of hydrogen. A fed-batch reactor configuration was tested at mesophilic temperature in laboratory experiments in order to improve the contact time and hydrogen mass transfer between the gas and liquid phases [13]. Effluent from an industrial biogas facility served as biocatalyst. The bicarbonate content of the effluent was depleted after some time, but the addition of stoichiometric carbon dioxide sustained hydrogen conversion for an extended period of time and prevented a pH shift. The microbial community generated biogas from the added α-cellulose substrate with concomitant hydrogen conversion, but the organic substrate did not facilitate hydrogen consumption. The fed-batch operational mode allowed a fourfold increase in volumetric hydrogen load and a 6.5-fold augmentation of the methane formation rate relative to the continuous stirred-tank reactor configuration. Acetate was the major by-product of the reaction.

A general strategy can be proposed on the basis of the results reported above to utilize the microbial community formed in the biogas reactor for the efficient conversion of hydrogen to biomethane as part of the power-to-gas principle. The studies demonstrated that microbiological communities are very efficient catalysts to combine hydrogen and carbon dioxide to methane, a renewable energy carrier that has already been in use in human practice for many years as fossil natural gas.

2. Utilization of biologically produced methane

Extensive reviews have been published on the energetic utilization of biogas, i.e. as an energy carrier for heat, electricity and transportation fuel [14]; therefore, these topics are not covered in this review. Exploitation of biologically produced methane in the chemical and fermentation industry incorporated into various biorefinery concepts represents an exciting new avenue of biotechnology leading to the realization of a circular economy strongly advocated in the EU recently [15]. Two aspects will be discussed in the following sections: alternative and more advanced use of biomethane to produce chemicals of high added value and the mitigation of undesired and uncontrolled biological methane emission in the atmosphere.

2.1. Biomethane is a platform substrate for the (bio)chemical industry

Methane constitutes the central component of natural gas, accounting for 80–90% of the raw natural gas. Methane can also be found, along with ethane as the major components, in shale gas, where the hydrocarbons are trapped within (shale) rock formations. As a fuel, methane could be thought of as an unbeatable source, since it provides the highest value of mass heat (ca. 56 kJ/g) compared with other hydrocarbons. Its general use as a raw material for chemical synthesis is reduced to a process in which methane is completely decomposed. The industrial use of methane consists of its conversion into synthesis gas (syngas), a mixture of carbon monoxide and molecular hydrogen that is further employed to produce methanol or in the Fischer–Tropsch process to give synthetic fuels. The availability of large amounts of methane makes this hydrocarbon a potential candidate as the feedstock for C1 chemistry. Unfortunately, its chemical inertness has meant that, after decades of efforts, such a goal yet constitutes a challenge for modern chemistry. From an ideal point of view, the best use for methane would be as a feedstock for C1 or C2 chemistry such as methanol (in an alternative method to that based on syngas) or acetic acid. This goal should start with the development of catalytic systems that would enhance methane reactivity to facilitate further conversion into higher molecules. The syngas term refers to any mixture of molecular hydrogen and carbon monoxide under appropriate conditions of pressure and temperature and the presence of solid catalysts. The process takes place at 5–30 atm and 800–950 °C, with conversions and yields of 70–75% (at 7 atm) [12,13]. Further discussion of this chemical route is beyond the

scope of this review.

From a biological perspective, methane represents a carbon and energy source for a group of bacteria known as methanotrophs. Methanotrophs are the only known significant biological sink for atmospheric methane and play a crucial role in reducing methane load. Methanotrophs use methane as their sole carbon source and directly convert methane into cellular compounds or transform it into a substrate that drives processes via methanotrophs or their syntrophic interaction with other microbes. Methanotrophs are aerobic proteobacteria. The proteobacteria phylum is divided into six classes according to rRNA sequences. Two classes contain methanotrophs: the alpha-proteobacteria and the gamma-proteobacteria.

The key enzyme allowing methane metabolism is methane monooxygenase (MMO), which occurs in a particulate form (pMMO) within the intracellular membrane or a soluble form (sMMO) within the cytoplasm. Copper availability plays a defining regulatory role with regard to sMMO expression, where sMMO synthesis is inhibited by higher Cu^{2+} concentrations. sMMO can be produced by various α- or γ-proteobacteria and has a much broader substrate range than pMMO. sMMO can cometabolize diverse types of hydrocarbons and halogenated organic compounds including aromatics. With the aid of biotechnology and genetic engineering, bacteria have been exploited for in situ bioremediation of a wide range of pollutants. Genetic engineering in methanotrophs may provide opportunities to exploit the unusual reactivity and broad substrate profile of MMO for maximum benefit in the field of remediation technologies and to manipulate the tolerance and degradation potential of methanotrophs against various organic and inorganic pollutants through the introduction of desired genes [17]. In addition, nitrogenase occurs in a broad range of methanotrophs allowing the use of N_2 as a nitrogen source [3].

The unique and ubiquitous nature and metabolic versatility of methanotrophs promote them as outstanding candidate platform organisms for biomethane-based biorefineries in a circular economy. Producing chemicals and fuels from biomethane expands the suite of products generated from biorefineries, municipalities and agricultural operations, with the potential to increase revenue and reduce greenhouse gas emissions [18]. By integrating this process into a conventional biorefinery, new opportunities for recycling and other cost reductions will become apparent.

2.2. Methanol production

The simplest bioconversion product of methane oxidation is methanol. Methanol is the first intermediate formed during the conversion of methane to carbon dioxide [19]. Methanol is liquid at ambient temperate and hence is more easily storable and transportable than methane. In vivo, methanol is quickly converted to formaldehyde in the methane oxidation to carbon dioxide. In addition, the reaction requires reduced coenzymes (NAD(P)H) and the cost of supplying enough reduced coenzymes adds to the problems to be solved. These hurdles should be overcome by genetic engineering or synthetic biology approaches before the process becomes industrially feasible. Essentially, the same is valid for the subsequent transitional products of biomethane oxidation, i.e. formaldehyde and formic acid.

2.3. Acids, epoxides, alcohols from biomethane

Biological production of value-added chemicals from biomethane represents a path to concurrently mitigate greenhouse gas emissions and utilize an abundant yet underutilized feedstock for the chemical industry. An eminent example of this successful and advanced approach has been reported recently [16,18]. Heterologous overexpression of L-lactate dehydrogenase in methanotrophic *Methylomicrobium buryatense* yielded significant yield and productivity of lactate, an industrial platform chemical and precursor of biodegradable polylactate for bioplastics production [20].

The broad substrate range of MMOs allows these enzymes to degrade soil contaminants as well as generate products such as 1- and 2-alcohols from C1–C8 n-alkanes, 1,2-epoxides from terminal alkenes and ethanol/ethanal from diethyl ether. Dalton and colleagues [3] studied the transformation of propylene (propene/methyl-ethylene) to propylene oxide (epoxy-propylene/epoxy-propane) using a methanotroph. A genetically modified methanotroph to produce farnesene using methane as the carbon source has been developed [3]. Farnesene is a global high-value commodity as it is a basic precursor for diesel, lubricants and specialty products (cosmetics, rubber and plastics). It is a sesquiterpene and occurs naturally in many plants including fruits such as apple, orange, mandarin, lime, pear and grapefruit, and also in ginger, nutmeg, basil and hops [21].

2.4. Biodiesel production

Another possible utilization of biomethane via methanotroph-based biotechnology is biodiesel production. Fei and coworkers [22] reviewed the efforts to use methanotrophs for biodiesel production. A two-stage fermentation process is preferred because excess lipid production should be induced by nitrogen- or phosphorus-limiting conditions and thereby by growth limitation.

2.5. Biopolymers from methane

More complex molecules can be synthesized from biomethane by methanotrophic bacteria as well. Extracellular polysaccharides (EPS) of various types are used in the pharmaceutical, textile, oil and food processing industries. Various polymeric materials can be obtained from algae, plants and EPS-producing bacteria. Methanotrophs also synthesize EPS from methane in remarkably high yield [23]. In addition to extracellular polymeric materials bacteria often produce intracellular polymers for energy and carbon storage purposes. The commonly synthesized storage polymers are various polyhydroxyalkanoates (PHA). These biopolymers are accumulated as water-insoluble granules within cells. Induction of PHA production is achieved when cells are supplied with copious amounts of carbon source under growth-limiting conditions, which is instigated by limiting nitrogen, phosphorus or sulfur nutrients [3]. The economy of PHA production is improved if the carbon source is supplied in the form of inexpensive biomethane. High-value polymeric materials find applications in the biomedical industry, e.g. in drug delivery, artificial organs or tissues slowly decomposing medical devices, etc. [22,23] and in the biodegradable packaging industry [24–26].

The last group of valuable products that can be produced from biomethane includes protein-based macromolecules. There are two major classes of valuable products to consider. Specialized enzymes, primarily the unique MMOs, in particular sMMO, are used for bioremediation and bioconversion reactions [3]. The other group of proteins is used in feed and food production. Methanotrophs were singled out as excellent single-cell protein (SCP) producers in the 1960s, when global protein production difficulties were predicted. Later the soya protein production boom temporarily provided a solution for the global protein shortage with the issue of mass production of edible protein from (bio)methane returns from time to time. Opponents of SCP utilization for feed or food purposes argue that SCP products contain too many nucleic acids, which may have negative effects on consumers. Technologies

to considerably eliminate this problem have been developed and a relatively inexpensive substrate in the form of (bio)methane may provide a competitive advantage to this technology [3]. In large-scale production of SCP, the selective pressure on fermentation by the sole C1 carbon source is an obvious advantage although infection by other bacteria should be carefully controlled and monitored because cell lysis always occurs and the released organic content may support the growth of undesired organisms.

The wide range of potential products from biomethane fermentation, as discussed above, convincingly demonstrates that biologically generated methane is a very useful commodity for various branches of the biochemical industry in addition to its value of being a renewable energy carrier.

3. Bio-methane mitigation

Biologically generated methane can also become be a global nuisance under conditions when its release into the atmosphere takes place in an uncontrolled process. After carbon dioxide, methane is the second largest contributing compound to global warming. Methane is not as abundant and decomposes faster than carbon dioxide; it is 25–28 times more effective in absorbing infrared light and thereby trapping heat in the atmosphere over a 100-year time period [27]. Methane has an average lifetime of 10 years in the atmosphere as it is primarily removed by conversion to carbon dioxide and water, whereas carbon dioxide has a lifetime of about 100 years. Reducing methane emission could therefore be an effective strategy to slow global warming within the next decades; 58–67% of methane released to the atmosphere each year originates from natural (wetlands, rice paddies) or anthropogenic sources.

Agriculture is responsible for approximately 10–12% of global anthropogenic greenhouse gas (GHG) emissions [28]. The contributions of enteric fermentation by ruminant animals and improper manure handling are the largest, accounting for nearly 80% of total agricultural emissions. Cattle emissions represent 60% of this value [29].

Enteric methane is a by-product of ruminant digestion, i.e. anaerobic microbiological decomposition of organic feedstock. The rumen is an anaerobic fermentation reactor, in which diverse and dense microbial communities live in symbiotic relationships and metabolites are exchanged to promote the growth of each member of the community by a complex process sometimes called “cross feeding” [30]. The anaerobic degradation process is similar to the one exploited in biogas reactors. The rumen is an anaerobic microbial ecosystem, which decomposes mostly complex plant carbohydrates to short-chain VFAs. VFAs are adsorbed through the rumen wall and used in energy metabolism and protein synthesis by the host ruminant [31]. During cross feeding, hydrogen-producing microbes and hydrogen-consuming methanogens cooperate in a tightly coupled process in order to facilitate the survival of both fibrinolytic fungi and bacteria, the hydrogen producers, and the hydrogenotrophic methanogens, the hydrogen consumers. The process occurs in a relatively short time frame (few days) compared with other anaerobic systems such as wetlands, rice paddies or biogas reactors (several weeks). VFA production is accompanied by generation of reducing equivalents, i.e. reduced cofactors, which need to be reoxidized by simultaneous hydrogen formation. Dissolved hydrogen inhibits ruminal anaerobic fermentation through negative feedback on VFA-producing acetogenic bacteria and eventually hinders the rate of subsequent feed decomposition, microbial growth and the synthesis of microbial protein [32]. Redox balances are maintained in the rumen by eliminating hydrogen in methane synthesis via methanogenic Archea present in the anaerobic microbial community. The challenge is to develop strategies to minimize methane production and increase milk, beef or wool production efficiency [33].

As a consequence of complex hydrogen and methyl group metabolism the degradation pathways diverge towards VFA production, methanogenesis, hydrogenation of lipids and microbial protein metabolism. The microbial community composition and its functional activity change dynamically, which contributes to the complexity and difficulty in predicting methane emissions and developing mitigation strategies.

Three major strategies have been proposed for mitigation of methane emission by the ruminant anaerobic microbial community and these will be briefly discussed in the next section.

3.1. Feeding management

The goal is to enhance propionate and/or decrease acetate production, which leads to reduced methanogenesis due to low hydrogen levels. Any dietary component or feeding management measure that results in promotion of propionate production is desired as propionate synthesis represents a hydrogen sink. The opposite is true for elevated acetate and butyrate fermentation. Hydrogenation of unsaturated fatty acids in lipid feedstock components also decreases the hydrogen levels available for methane formation [34]. High-quality diets, i.e. more digestible and containing more energy, usually contain more starch and less methane is produced due to accelerated microbial activity. Improved feed intake, high milk yields and low methane emission accompanied feeding with more digestible polymeric carbohydrates [35]. Soluble sugars, however, tend to divert fermentation to higher butyrate production [36]. Numerous other nutritional parameters have been studied, some with contradicting results. The daily dry matter intake, forage particle size, rumen pH, forage quality, harvesting and storage of plant materials all contribute to the metabolism of ruminants and thus have a direct but extremely complex effect on the composition and biological activity of rumen microbiota [37]. Improving our understanding of the interrelationships affecting overall rumen fermentation will lead to better means of manipulating anaerobic fermentation and methane emission control [36,37].

3.2. Direct inhibition of methanogenesis

Specific substances are searched for to reduce methanogen abundance or viability. This is apparently a greatly appealing idea in rumen emission research and numerous proposed solutions have been suggested.

Glucose is mainly metabolized through glycolysis to pyruvate in the rumen. Glycolysis and pyruvate oxidative decarboxylation to acetyl-CoA, which is the first step in acetate and butyrate formation, both result in the release of metabolic hydrogen. Reduced cofactors must then be reoxidized for fermentation to continue. In the typical ruminal fermentation, methanogenesis is the main route of cofactor reoxidation, with hydrogen transferred from fermentative bacteria, protozoa and fungi to methanogenic archaea mainly as hydrogen. However, the production of propionate, a useful fermentation product and the main glucose precursor for ruminants, competes with methane production for hydrogen [40]. Also, some hydrogen is incorporated into butyrate production from pyruvate and although butyrate production from hexoses results in net release of hydrogen, less hydrogen per mole of glucose is released from butyrate formation compared to acetate [41]. Methanogenesis inhibitors were classified [41,42] according to their known or presumed mode of action into: a.) pure chemical compounds changing methanogens or their hydrogen supply directly; b.) nitrate and nitro compounds, which apart from being toxic to

methanogens can also decrease methane production by competing as electron acceptors for hydrogen; c.) ionophores, which mainly inhibit organisms involved in producing hydrogen; d.) oils, plant extracts and antiprotozoal, antibacterial agents, which can inhibit methanogens directly.

3.2.1. Chemical compounds

3.2.1.1. Propionate precursors. Starch is readily fermented by rumen microbiota. As a consequence of starch fermentation the acetate/propionate ratio and the pH in the rumen are decreased. There are two pathways leading to propionate fermentation. The pyruvate-lactate-acetyl CoA-propionate metabolic route is less intensively used by ruminants than the pyruvate-malate-fumarate-succinate-propionate pathway. This is particularly true if the ratio of forage increases in the diet [43]. An elevated lactate level would decrease the pH, which has selective actions on rumen microorganisms, especially on cellulolytic bacteria. Supplementation of malate or fumarate is a possible way to reduce methane production via the more active and hydrogen-consuming malate-fumarate-succinate-propionate route. Fumarate is an intermediate compound of this pathway in the rumen and is reduced to succinate by fumarate reductase (EC. 1.3.99.1). Since hydrogen is utilized to reduce fumarate, fumarate-reducing (dissimilating) bacteria and methanogenic archaea will compete for hydrogen in the presence of fumarate [43,44]. In fact, reduction of methane production by fumarate supplementation was observed both in vitro and in vivo [45,46].

3.2.1.2. Methanogen inhibitors. Several chemicals have been tested as specific inhibitors of methanogens to decrease methane emission by ruminants. 2-bromo-ethane sulfonate (BES) is among the first investigated inhibitors. Coenzyme M (2-mercapto-ethane sulfonate) is a cofactor involved in the final step of methanogenesis, transferring a methyl group to methyl-coenzyme M reductase (MCR). MCR is irreversibly inhibited by BES, a structural analogue of coenzyme M, which makes BES a widely used specific inhibitor of methanogenesis [47]. BES serves as a fermentation substrate or terminal electron acceptor in anaerobic mixed cultures. The mechanism of BES degradation is most likely the reduction of the sulfonate moiety by sulfate reducers. *Desulfovibrio*-like organisms were found to be enriched in sulfate-free enrichment cultures containing accumulated BES sulfide. Sulfides are toxic for both the microbial community and the host; therefore, BES does not seem suitable for large-scale control of ruminant methanogenesis. In addition, as a result of methanogenesis inhibition, hydrogen would accumulate, which suppresses rumen fermentation by inhibiting acetogenesis, i.e. formation of VFAs and hydrogen.

Bromochloromethane (BCM) reacts with reduced vitamin B_{12} and thus inhibits B_{12}-mediated methyl group transfer in methanogens [48,49]. Its action on methane reduction in vitro was confirmed, but the compound is a volatile, strongly ozone-depleting GHG and therefore its use seemed impractical. Its cyclodextrin derivative (BCM-CD), however, apparently avoided the volatility problem and maintained biological activity [50]. The physiological effects associated with the administration of the compound on host animals and those consuming their products remain to be established. BCM-CD was shown to shift VFA metabolism in the rumen towards propionate by decreasing the acetate to propionate ratio, which confirms previous reasoning [40–42,51] concerning the importance of hydrogen sinks in methane emission mitigation. The long-term effects of these compounds are still uncertain and fear of their potential carcinogenic nature bars their adoption in animal nutrition.

Propane thiosulfate (PTS), and diallyl disulfide (DDS) were similarly effective candidates to limit rumen methanogenesis [52], but these ingredients of garlic provide a specific taste and odor, which are unpleasant for many milk and beef consumers.

3.2.1.3. Nitrate, sulfate and nitro compounds. Reduction of nitrate in the rumen is one of the important pathways yielding ammonia, which is utilized by bacteria as a nitrogen source. Conversion of 1 mol of nitrate to ammonia consume 4 mol of hydrogen, hence nitrate appears an excellent hydrogen sink. Dietary nitrate has been considered promising in methane emission mitigation in spite of the bitter taste of nitrate, which may cause lower feed intake. High concerns about nitrate stem from the fact that nitrate is reduced to ammonia in two steps. Unfortunately, the first reaction, i.e. reduction of nitrate to nitrite, is much faster that the subsequent nitrite reduction. Nitrite is absorbed across the rumen wall into the blood circulation, where it irreversibly binds to hemoglobin to form methemoglobin. Methemoglobin is incapable of carrying oxygen; nitrite is therefore toxic for the host animal [53]. In addition, a shift in VFA concentrations from propionate to acetate has been observed upon feeding dairy cows with a nitrate-rich diet. Thus nitrite formation effectively competes with the fumarate-succinate-propionate pathway and acts against methane emission mitigation via this route and has a detrimental effect on host nutrition.

Sulfate also acts as a terminal electron acceptor and sulfate reducers can use hydrogen at lower partial pressures thus making them able to outcompete methanogens when hydrogen levels are low. However, sulfur tolerance in the diet is relatively low, due to the formation of toxic hydrogen sulfide, suggesting little potential as a method to reduce methane emissions.

An in silico screening approach [54] identified some nitrooxy carboxylic acids with potential to dock into the active site of methyl-CoM-reductase, the enzyme catalyzing the final step in the reduction of carbon dioxide to methane by methanogenic archaea. 3-Nitrooxypropanol (3-NOP) has been selected and tested under various experimental conditions. Inhibition of methanogenesis both in vitro and in vivo in sheep was apparent [55]. In dairy cows, however, the effect was transitory when 3-NOP was delivered into the rumen via rumen fistula [56]. This suggested that the compound may had been washed out of the rumen, or metabolized or absorbed. Milk yield and milk fat were not affected but milk protein was elevated. In contrast, when 3-NOP was mixed into the daily diet and therefore consumed by the lactating cows continuously, methane emissions decreased significantly [39,57,58]. Interestingly, the daily dry matter intake or milk or milk components did not change, but the cows receiving 3-NOP gained more body weight. Acetate production relative to propionate decreased, pointing to the importance of hydrogen sink generation as a tool to decrease methane emission by ruminants [40–42,51].

3.2.1.4. Ionophores. Ionophores are highly lipophilic polyethers that accumulate in cell membranes and facilitate rapid ion exchange across the membrane [59]. Monensin is a polyether antibiotic isolated from *Streptomyces cinnamonensis* [60]. Its antibacterial properties are the result of its ability to transport metals through cellular or subcellular membranes. Monensin is inhibitory for protozoa and Gram-positive bacteria such as ruminococci, streptococci and lactobacilli but not for Gram-negative bacteria, and therefore leads to a rumen microbiota producing more propionate and less acetate. Reduction of methane emissions upon monensin treatment is not due to a reduction of methanogens but is more likely due to the development of an alternative hydrogen-consuming pathway such as propionate- and succinate-producing bacterial activities. Monensin has a moderate methane emission mitigating effect in animals fed high-grain or mixed-grain forage diets [39]. These and similar antibiotics are not

recommended for large-scale use due to the resistance development hazard [59].

3.3. Plant secondary metabolites

3.3.1. Tannins and saponins

Tannins are diverse polyphenolic compounds. Their multiple phenolic hydroxyl groups help to form complexes with proteins, polysaccharides, amino acids and various metal ions [61]. Saponins are natural detergents. These compounds form complexes preferentially with sterols in protozoal cell membranes and selectively inhibit some bacteria as well. Rumen protozoa live in symbiosis with methanogenic archaea; therefore, their eradication leads to reduced methanogenesis. Although a high amount of tannin-rich plant forage may downgrade methane emissions, a high dietary concentration of tannins from chestnut, mimosa, quebracho and sumach negatively affected feed digestibility and animal performance [39].

As for tannins, the effect of saponions is concentration dependent. The main commercial source of saponions is extracts of yucca plant and the results are ambiguous [61]. In studies reporting a significant reduction of methanogenesis an increase in propionate production and decrease in protozoal number was established [62]. Nevertheless, the results of supplying various saponin-containing components in the diet were not consistent and indicated that the correlation between methanogenesis and methanogen-harboring protozoa is weak [61,63].

3.3.2. Essential oils

Plant-derived essential oils may be a useful tool to improve the efficiency of nutrient utilization and animal performance. They have a wide range of antibacterial activities. Unfortunately, long-term studies suggested that the benefits associated with the inclusion of essential oils in the ruminant diet diminish over time due to adaptation of the microbial community. In addition the chemical composition of “plant essential oils” is rather vague and the nutritional and methane emission results are not conclusive. Essential oils include a mixture of natural or natural-identical compounds such as thymol, eugenol, vanillin, guaiacol and limonene, among others [64]. Addition of sunflower, linseed, coconut or palm kernel oils and other lipids to the regular diet gave similarly confounding results [65,66]. From about 500 plant species two were selected to study their methane mitigation effect, i.e. Italian plumeless thistle (*Carduus pycnocephalus*) and Sikkim rhubarb (*Rheum nobile*), to test their methane mitigation activity [30]. On a high-forage diet a pronounced inhibition of methanogenesis was observed, while less inhibition was noted on a high-concentrate diet. The reduction of methane emissions was not accompanied by enhanced feed utilization, such as propionate fermentation.

A number of plant secondary metabolites have been shown to possess methanogenesis-inhibiting property as feed additives in ruminants [67]. In this study, leaves of *Carduus*, rhubarb and the bulb of garlic have been selected as the most effective methane mitigation candidates. The antibacterial nature of these plants is widely known, but their ingredients accumulate in the milk and meat of ruminants and provide an unpleasant flavor. Nevertheless, in vitro studies corroborated their beneficial effect on diminishing methanogenesis [68].

At any rate, stimulation of propionate production seems to be the best alternative hydrogen sink to methanogenesis in the rumen [38–40,67,69]. Our understanding of rumen microbiota and their association to the host's metabolism is still incomplete; elucidation of microbial diversity and interrelationships is fundamental for the successful management of rumen fermentation towards more

Table 1
Summary of strategies for methane emission mitigation by ruminants.

Strategy				Inhibition mechanism	Advantage	Disadvantage
Feeding management				H_2 sink generation: propionate synthesis, unsaturated fatty acids	Improved feed intake, productivity	Difficult to manage; not fully understood
Direct inhibition	Chemical compounds	Propionate precursors	Lactate	Shift towards propionate biosynthesis	Easily manageable	Decreasing pH; Expensive
			Malate, fumarate			Expensive
		Methanogen inhibitors	BES	Inhibition of methyl-CoM-reductase	Direct, specific	Toxic decomposition products
			BCM	B_{12} inhibition		GHG compound; Uncertain decomposition
			PTS, DDS	Bacteriocid	Biocompatible	Unpleasant taste in milk and meat
		Nitrate, sulfate and nitro compounds	NO_3^-	H_2 sink	Inexpensive	Toxic nitrite and ammonia
			SO_4^{2-}			Toxic sulfides
			3-NOP	Inhibition of methyl-CoM-reductase	Specific	Expensive
		Ionophores	Monensin	Cell membrane disruption	Inhibits protozoa and Gram positive bacteria	Antibiotic, not allowed to use
	Plant metabolites	Tannins		Protozoa inhibitor, inhibits methanogens	Effective, natural compounds	Negative effect on feed intake and animal performance
		Saponins		Detergent, Cell membrane disruption		
		Essential oils		Antibacterial natural extracts	Effective in some cases	Unpleasant taste in milk and meat
Breeding and genetics				Selection based on animal control of rumen microbiota	No chemical or biological contamination	Requires long time; Unknown, unexplored

efficient feed utilization and reduced methane emission. Dairy cows that grazed on lush pasture, however, showed limited effects of essential oil on ruminal fermentation, milk production and milk composition [70] indicating that natural grazing on good pasture provides the ingredients of balanced rumen fermentation.

3.4. Breeding and genetics

This concept aims to select animals and herds having improved feed utilization efficiency and diminished methane production. A two-way interaction between the host ruminant and its microbiota has been assumed for some time [71,72]. If the animal itself has some control over its ruminal microbial community and the trait is heritable, low methane-emitting phenotypes may lead to breeding of ruminants with a smaller carbon footprint while maintaining milk or beef productivity. Lower methane emission may also result in higher energy retention by the animal [31]. A possible mechanism explaining methane emission differences between animals is based on the amount of time that feed particles are retained in the rumen. Longer retention times apparently lead to higher methane yields [73]. Difference in the passage rate affects ruminal hydrogen levels [40] and less hydrogen formation by the fermentative bacteria results in less methane production. Particle retention time is known to be a heritable trait; therefore, selection of low methane-emitting, i.e. higher passage rate of particles, genetic lines may become feasible. The total methanogen richness in the rumen contents did not differ between the feed efficiency of animals, indicating that the composition of the methanogenic community was the important difference [73]. The family *Succinivibrionaceae* has been suggested as a biomarker indicating a low methane emission phenotype in ruminants [31]. The rumen, however, is still a largely unexplored environment containing many uncharacterized microbes, which could be of significant interest in agricultural and environmental management practices. The genetic influences of the host on the microbial community are affected by various biological factors. A simple example invokes saliva production, which is partly genetically determined, and affects rumen pH, a functional parameter influencing rumen microbiota composition. Variations in the intensity of ruminal contractions and rate of passage likewise contribute to the balanced composition of rumen microbiota [74]. The key characteristics of the various methane mitigation methods are summarized in Table 1.

Various studies showed that the abundance of microbial genes is more closely associated with metabolism than the abundance of the microbial community and host genetic influence is significant on those traits. Understanding the complex networks of interactions would eventually lead to exploitation of the genetic link between hosts and their microbiota and successful mitigation of methane emissions of ruminant origin.

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References

[1] G. Gahleitner, Hydrogen from renewable electricity: an international review of power-to-gas pilot plants for stationary applications, Int. J. Hydrogen Energy 38 (2013) 2039–2061, http://dx.doi.org/10.1016/j.ijhydene.2012.12.010.
[2] N. Thakur, A. Khardenavis, H.J. Purohit, Recent Advances in Factors and Methods for Stimulation of Biomethane Production, 2015, pp. 3–13.
[3] P.J. Strong, S. Xie, W.P. Clarke, Methane as a resource: can the methanotrophs add value? Environ. Sci. Technol 49 (2015) 4001–4018, http://dx.doi.org/10.1021/es504242n.
[4] B. Demirel, P. Scherer, The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review, Rev. Environ. Sci. Biotechnol 7 (2008) 173–190, http://dx.doi.org/10.1007/s11157-008-9131-1.
[5] F. Zhang, Y. Zhang, Y. Chen, K. Dai, M.C.M. van Loosdrecht, R.J. Zeng, Simultaneous production of acetate and methane from glycerol by selective enrichment of hydrogenotrophic methanogens in extreme-thermophilic (70°C) mixed culture fermentation, Appl. Energy 148 (2015) 326–333, http://dx.doi.org/10.1016/j.apenergy.2015.03.104.
[6] Z. Bagi, N. Ács, B. Bálint, L. Horváth, K. Dobó, K.R. Perei, G. Rákhely, K.L. Kovács, Biotechnological intensification of biogas production, Appl. Microbiol. Biotechnol 76 (2007) 473–482, http://dx.doi.org/10.1007/s00253-007-1009-6.
[7] Y. ping Hou, D. cong Peng, X. dong Xue, H. ye Wang, L. ying Pei, Hydrogen utilization rate: a crucial indicator for anaerobic digestion process evaluation and monitoring, J. Biosci. Bioeng 117 (2014) 519–523, http://dx.doi.org/10.1016/j.jbiosc.2013.10.006.
[8] J.C. Lee, J.H. Kim, W.S. Chang, D. Pak, Biological conversion of CO 2 to CH 4 using hydrogenotrophic methanogen in a fixed bed reactor, J. Chem. Technol. Biotechnol 87 (2012) 844–847, http://dx.doi.org/10.1002/jctb.3787.
[9] M. Alimahmoodi, C.N. Mulligan, Anaerobic bioconversion of carbon dioxide to biogas in an upflow anaerobic sludge blanket reactor, J. Air Waste Manag. Assoc 58 (2008) 95–103, http://dx.doi.org/10.3155/1047-3289.58.1.95.
[10] I. Bassani, P.G. Kougias, L. Treu, I. Angelidaki, Biogas upgrading via hydrogenotrophic methanogenesis in two-stage continuous stirred tank reactors at mesophilic and thermophilic conditions, Environ. Sci. Technol (2015), http://dx.doi.org/10.1021/acs.est.5b03451.
[11] G. Luo, I. Angelidaki, Integrated biogas upgrading and hydrogen utilization in an anaerobic reactor containing enriched hydrogenotrophic methanogenic culture, Biotechnol. Bioeng 109 (2012) 2729–2736, http://dx.doi.org/10.1002/bit.24557.
[12] G. Luo, S. Johansson, K. Boe, L. Xie, Q. Zhou, I. Angelidaki, Simultaneous hydrogen utilization and in situ biogas upgrading in an anaerobic reactor, Biotechnol. Bioeng (2011), http://dx.doi.org/10.1002/bit.24360.
[13] M. Szuhaj, N. Acs, R. Tengolics, A. Bodor, G. Rakhely, K.L. Kovacs, Z. Bagi, Conversion of H2 and CO2 to CH4 and acetate in fed-batch biogas reactors by mixed biogas community: a novel route for the power-to-gas concept, Biotechnol. Biofuels 9 (2016) 102, http://dx.doi.org/10.1186/s13068-016-0515-0.
[14] European Biogas Association, Biomethane in Transport, Bussels, 2016.
[15] A. Caballero, P.J. Pérez, Methane as raw material in synthetic chemistry: the final frontier, Chem. Soc. Rev 42 (2013) 8809, http://dx.doi.org/10.1039/c3cs60120j.
[16] G.A. S.P.G. Olah, A. Goeppert, Beyond Oil and Gas: the Methanol Economy, second ed., Wiley-VCH, Weinheim, 2009.
[17] A.W. Puri, S. Owen, F. Chu, T. Chavkin, D.A.C. Beck, M.G. Kalyuzhnaya, M.E. Lidstrom, Genetic tools for the industrially promising methanotroph Methylomicrobium buryatense, Appl. Environ. Microbiol 81 (2015) 1775–1781, http://dx.doi.org/10.1128/AEM.03795-14.
[18] C.A. Henard, H. Smith, N. Dowe, M.G. Kalyuzhnaya, P.T. Pienkos, M.T. Guarnieri, Bioconversion of methane to lactate by an obligate methanotrophic bacterium, Sci. Rep 6 (2016) 21585, http://dx.doi.org/10.1038/srep21585.
[19] J.G. Ferry, Fundamentals of methanogenic pathways that are key to the biomethanation of complex biomass, Curr. Opin. Biotechnol 22 (2011) 351–357, http://dx.doi.org/10.1016/j.copbio.2011.04.011.
[20] N.L. Haag, C. Grumaz, F. Wiese, P. Kirstahler, W. Merkle, H.J. Nägele, K. Sohn, T. Jungbluth, H. Oechsner, Advanced green biorefining: effects of ensiling treatments on lactic acid production, microbial activity and supplementary methane formation of grass and rye, Biomass Convers. Biorefinery 6 (2016) 197–208, http://dx.doi.org/10.1007/s13399-015-0178-2.
[21] J. Šobotník, R. Hanus, B. Kalinová, R. Piskorski, J. Cvačka, T. Bourguignon, Y. Roisin, (E,E)-α-farnesene, an alarm pheromone of the termite Prorhinotermes canalifrons, J. Chem. Ecol 34 (2008) 478–486, http://dx.doi.org/10.1007/s10886-008-9450-2.
[22] Q. Fei, M.T. Guarnieri, L. Tao, L.M.L. Laurens, N. Dowe, P.T. Pienkos, Bioconversion of natural gas to liquid fuel: opportunities and challenges, Biotechnol. Adv 32 (2014) 596–614, http://dx.doi.org/10.1016/j.biotechadv.2014.03.011.
[23] I.P. Malashenko, T.P. Pirog, V.A. Romanovskaia, I.G. Sokolov, T.A. Gringerg, Search for methanotrophic producers of exopolysaccharides, Prikl. Biokhim. Mikrobiol 37 (2001) 702–705, http://dx.doi.org/10.1023/A:1012307202011.
[24] C.W. Pouton, S. Akhtar, Biosynthetic polyhydroxyalkanoates and their potential in drug delivery, Adv. Drug Deliv. Rev 18 (1996) 133–162, http://dx.doi.org/10.1016/0169-409X(95)00092-L.
[25] Sinskey Brigham, Applications of polyhydroxyalkanoates in the medical industry, Int. J. Biotechnol. Wellness Ind (2012) 53–60, http://dx.doi.org/10.6000/1927-3037.2012.01.01.03.
[26] E. Bugnicourt, P. Cinelli, A. Lazzeri, V. Alvarez, Polyhydroxyalkanoate (PHA): review of synthesis, characteristics, processing and potential applications in packaging, Express Polym. Lett 8 (2014) 791–808, http://dx.doi.org/10.3144/

expresspolymlett.2014.82.
[27] S. T.P. Schwietzke, O.A. Sherwood, L.M.P. Bruhwiler, J.B. Miller, G. Etiope, E.J. Dlugokencky, S.E. Michel, V.A. Arling, B.H. Vaughn, J.W.C. White, Upward revision of global fossil fuel methane emissions based on isotope database, Nature 538 (2016) 88–91, http://dx.doi.org/10.1038/nature19797.
[28] J. Broucek, Production of methane emissions from ruminant Husbandry: A Review, 2014, pp. 1482–1493.
[29] C. Opio, P. Gerber, A. Mottet, A. Falcucci, G. Tempio, M. MacLeod, T. Vellinga, Greenhouse Gas Emissions from Ruminant Supply Chains, 2013.
[30] Y. Kobayashi, Abatement of Methane Production from Ruminants: Trends in the Manipulation of Rumen Fermentation*, vol. 23, 2010, pp. 410–416.
[31] R. John Wallace, John A. Rooke, Nest McKain, Carol-Anne Duthie, Jimmy J. Hyslop, David W. Ross, Anthony Waterhouse, Mick Watson, Rainer Roehe, The rumen microbial metagenome associated with high methane production in cattle, BMC Genomics 16 (2015) 839, http://dx.doi.org/10.1186/s12864-015-2032-0.
[32] D.O. Krause, T.G. Nagaraja, A.D.G. Wright, T.R. Callaway, Board-invited review: rumen microbiology: Leading the way in microbial ecology 1 (2) (2014) 331–341, http://dx.doi.org/10.2527/jas2012-5567.
[33] L.G.R. C.C. Pereira, F.S. Machado, M.M. Campos, R.G. Júnior, T.R. Tomich, L.G. Reis, Enteric methane mitigation strategies in ruminants: a review, Rev. Colomb. Ciencias Pecu 28 (2015) 124–143, http://dx.doi.org/10.17533/udea.rccp.v28n2a02.
[34] C. Grainger, K.A. Beauchemin, Can enteric methane emissions from ruminants be lowered without lowering their production? Anim. Feed Sci. Technol 166–167 (2011) 308–320, http://dx.doi.org/10.1016/j.anifeedsci.2011.04.021.
[35] L. Abecia, G. Cantalapiedra-hijar, I. Martín-garcía, D.R. Yáñez-ruiz, P. Amaro, M.R.G. Maia, R.J. Dewhurst, A.J.M. Fonseca, A.R.J. Cabrita, R. Bodas, N. Prieto, R. García-gonzález, S. Andrés, F.J. Giráldez, S. López, B.M. Buddle, M. Denis, G.T. Attwood, E. Altermann, P.H. Janssen, R.S. Ronimus, C.S. Pinares-patiño, S. Muetzel, D.N. Wedlock, Y. Chen, J.J. Cheng, K.S. Creamer, A. Cieslak, A. Stochmal, W. Oleszek, R. Environ, S. Biotechnol, A.J. Flores, A.D. Garciarena, J.M. Hernández, K.A. Beauchemin, D. Colombatto, I. Giannenas, J. Skoufos, C. Giannakopoulos, M. Wiemann, O. Gortzi, S. Lalas, I. Kyriazakis, C. Grainger, K.A. Beauchemin, J.L. Haisan, K. Hattori, H.M.Ā.G. Henderson, G.E. Naylor, S.C. Leahy, P.H. Janssen, M. Tavendale, P.H. Janssen, S.E. Hook, G. Wright, B.W. Mcbride, A.N. Hristov, J. Oh, J.L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H.P.S. Makkar, P.H. Janssen, K. Joblin, M. Chavarot, R. Roux, G.E. Naylor, F. Michallon, L.K. Jr, P. Williams, R.J. Schmidt, W. Hu, J.R. Knapp, G.L. Laur, P.A. Vadas, W.P. Weiss, J.M. Tricarico, Y.Y. Kobayashi, D.O. Krause, T.G. Nagaraja, A.D.G. Wright, T.R. Callaway, S.Y. Lee, S.H. Yang, W.S. Lee, H.S. Kim, D.E. Shin, J.K. Ha, L. Lillis, B. Boots, D.A. Kenny, K. Petrie, T.M. Boland, N. Clipson, E.M. Doyle, B. Lin, Y. Lu, A.Z.M. Salem, J.H. Wang, Q. Liang, J.X. Liu, P. Maria, B. Piet, R.D. Hall, J. Beekwilder, C. Martin, D.P. Morgavi, M. Doreau, M. Mitsumori, T. Shinkai, A. Takenaka, O. Enishi, K. Higuchi, Y.Y. Kobayashi, I. Nonaka, N. Asanuma, S.E. Denman, C.S. Mcsweeney, W. Sun, P. Murray, C.J. Newbold, J. Of, D. Science, A.K. Patra, J. Saxena, Z. Yu, C.K. Reynolds, D.J. Humphries, P. Kirton, M. Kindermann, S. Duval, W. Steinberg, R.J. Schmidt, V. Siriwongrungson, R.J. Zeng, I.A.Ā, E.M. Ungerfeld, Z. Zhou, Q. Meng, Z. Yu, A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows, J. Dairy Sci 97 (2014) 24–32, http://dx.doi.org/10.3168/jds.2008-1402.
[36] J.A. Rooke, R.J. Wallace, C.A. Duthie, N. McKain, S.M. de Souza, J.J. Hyslop, D.W. Ross, T. Waterhouse, R. Roehe, Hydrogen and methane emissions from beef cattle and their rumen microbial community vary with diet, time after feeding and genotype, Br. J. Nutr 112 (2014) 398–407, http://dx.doi.org/10.1017/S0007114514000932.
[37] J.R. Knapp, G.L. Laur, P.A. Vadas, W.P. Weiss, J.M. Tricarico, Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions, J. Dairy Sci 97 (2014) 3231–3261, http://dx.doi.org/10.3168/jds.2013-7234.
[38] E. Jami, B.A. White, I. Mizrahi, Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency, PLoS One 9 (2014), http://dx.doi.org/10.1371/journal.pone.0085423.
[39] A.N. Hristov, J. Oh, J.L. Firkins, J. Dijkstra, E. Kebreab, G. Waghorn, H.P.S. Makkar, SPECIAL TOPICS — mitigation of methane and nitrous oxide emissions from animal operations: I, A Rev. enteric methane Mitig. options 1 (2014), http://dx.doi.org/10.2527/jas2013-6583.
[40] P.H. Janssen, Author ' S Personal Copy Influence of Hydrogen on Rumen Methane Formation and Fermentation Balances through Microbial Growth Kinetics and Fermentation Thermodynamics, (n.d.). doi:10.1016/j.anifeedsci.2010.07.002.
[41] E.M. Ungerfeld, Limits to dihydrogen incorporation into electron sinks alternative to methanogenesis in ruminal fermentation, Front. Microbiol 6 (2015) 1–14, http://dx.doi.org/10.3389/fmicb.2015.01272.
[42] E.M. Ungerfeld, Shifts in metabolic hydrogen sinks in the methanogenesis-inhibited ruminal fermentation: a meta-analysis, Front. Microbiol 6 (2015) 1–17, http://dx.doi.org/10.3389/fmicb.2015.00037.
[43] M. Mitsumori, W. Sun, Control of Rumen Microbial Fermentation for Mitigating Methane Emissions from the Rumen *, vol. 21, 2008, pp. 144–154.
[44] K. Hattori, H. Matsui, Diversity of fumarate reducing bacteria in the bovine rumen revealed by culture dependent and independent approaches, Anaerobe 14 (2008) 87–93, http://dx.doi.org/10.1016/j.anaerobe.2007.12.002.
[45] T.A. Wood, R.J. Wallace, A. Rowe, J. Price, D.R. Yáñez-Ruiz, P. Murray, C.J. Newbold, Encapsulated fumaric acid as a feed ingredient to decrease ruminal methane emissions, Anim. Feed Sci. Technol 152 (2009) 62–71, http://dx.doi.org/10.1016/j.anifeedsci.2009.03.006.
[46] L. Mamuad, S.H. Kim, C.D. Jeong, Y.J. Choi, C.O. Jeon, S.S. Lee, Effect of fumarate reducing bacteria on in vitro rumen fermentation, methane mitigation and microbial diversity, J. Microbiol 52 (2014) 120–128, http://dx.doi.org/10.1007/s12275-014-3518-1.
[47] W.E. Balch, R.S. Wolfe, Transport of coenzyme M (2-mercaptoethanesulfonic acid) in Methanobacterium ruminantium, J. Bacteriol 137 (1979) 264–273.
[48] S.Y. Lee, S.H. Yang, W.S. Lee, H.S. Kim, D.E. Shin, J.K. Ha, Effect of 2-Bromoethanesulfonic Acid on in Vitro Fermentation Characteristics and Methanogen Population *, vol. 22, 2009, pp. 42–48.
[49] G. Goel, H.P.S. Makkar, K. Becker, Inhibition of methanogens by bromochloromethane: effects on microbial communities and rumen fermentation using batch and continuous fermentations, Br. J. Nutr 101 (2009) 1484–1492, http://dx.doi.org/10.1017/S0007114508076198.
[50] S.E. Denman, G.M. Fernandez, T. Shinkai, M. Mitsumori, C.S. McSweeney, Metagenomic analysis of the rumen microbial community following inhibition of methane formation by a halogenated methane analog, Front. Microbiol 6 (2015) 1–12, http://dx.doi.org/10.3389/fmicb.2015.01087.
[51] E.M. Ungerfeld, R.A. Kohn, R.J. Wallace, C.J. Newbold, A meta-analysis of fumarate effects on methane production in ruminal batch cultures, J. Anim. Sci 85 (2007) 2556–2563, http://dx.doi.org/10.2527/jas.2006-674.
[52] G. Martínez-Fernández, L. Abecia, A.I. Martín-García, E. Ramos-Morales, S.E. Denman, C.J. Newbold, E. Molina-Alcaide, D.R. Yáñez-Ruiz, Response of the rumen archaeal and bacterial populations to anti-methanogenic organosulphur compounds in continuous-culture fermenters, FEMS Microbiol. Ecol 91 (2015) 1–11, http://dx.doi.org/10.1093/femsec/fiv079.
[53] C. Yang, J.A. Rooke, I. Cabeza, R.J. Wallace, Nitrate and inhibition of ruminal methanogenesis: microbial ecology, obstacles, and opportunities for lowering methane emissions from ruminant livestock, Front. Microbiol 7 (2016) 1–14, http://dx.doi.org/10.3389/fmicb.2016.00132.
[54] R.A. Friesner, J.L. Banks, R.B. Murphy, T.A. Halgren, J.J. Klicic, D.T. Mainz, Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy, J. Med. Chem 47 (2004) 1750–1759, http://dx.doi.org/10.1021/jm0306430.
[55] G. Y.R. Martinez-Fernández, L. Abecia, A. Arco, G. Cantalapiedra-Hijar, A.I. Martín-Garcia, E. Molina-Alcaide, M. Kindermann, S. Duval, Effects of ethyl-3-nitrooxy propionate and 3-nitrooxypropanol on ruminal fermentation, microbial abundance, and methane emission in sheep, J. Dairy Sci 97 (2014) 3790–3799, http://dx.doi.org/10.3168/jds.2013-7398.
[56] C.K. Reynolds, D.J. Humphries, P. Kirton, M. Kindermann, S. Duval, W. Steinberg, Effects of 3-nitrooxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows, J. Dairy Sci 97 (2014) 3777–3789, http://dx.doi.org/10.3168/jds.2013-7397.
[57] J.L. Haisan, Effects of feeding 3-nitrooxypropanol to lactating dairy cows on methane emissions, animal performance and rumen fermentation by, (2014).
[58] A.N. Hristov, J. Oh, F. Giallongo, T.W. Frederick, M.T. Harper, H.L. Weeks, A.F. Branco, P.J. Moate, M.H. Deighton, S.R.O. Williams, M. Kindermann, S. Duval, An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production, Proc. Natl. Acad. Sci 112 (2015) 10663–10668, http://dx.doi.org/10.1073/pnas.1504124112.
[59] J.B. Russell, A.J. Houlihan, Ionophore resistance of ruminal bacteria and its potential impact on human health, FEMS Microbiol. Rev 27 (2003) 65–74, http://dx.doi.org/10.1016/S0168-6445(03)00019-6.
[60] D. Łowicki, A. Huczyński, D. Łowicki, A. Huczyński, Ł.D. Owicki, Huczyń, A. Ski, Structure and antimicrobial properties of monensin a and its derivatives: summary of the achievements, Biomed. Res. Int. 2013 (2013) 1–14, http://dx.doi.org/10.1155/2013/742149.
[61] G. Goel, H.P.S. Makkar, Methane mitigation from ruminants using tannins and saponins, Trop. Anim. Health Prod 44 (2012) 729–739, http://dx.doi.org/10.1007/s11250-011-9966-2.
[62] A.N. Hristov, T.A. McAllister, F.H. Van Herk, K.J. Cheng, C.J. Newbold, P.R. Cheeke, Effect of Yucca schidigera on ruminal fermentation and nutrient digestion in heifers, J. Anim. Sci 77 (1999) 2554–2563 doi:/1999.7792554x.
[63] A.K. Patra, J. Saxena, The Effect and Mode of Action of Saponins on the Microbial Populations and Fermentation in the Rumen and Ruminant Production, 2009, pp. 204–219, http://dx.doi.org/10.1017/S0954422409990163.
[64] I. Giannenas, J. Skoufos, C. Giannakopoulos, M. Wiemann, O. Gortzi, S. Lalas, I. Kyriazakis, Effects of essential oils on milk production, milk composition, and rumen microbiota in Chios dairy ewes, J. Dairy Sci 94 (2011) 5569–5577, http://dx.doi.org/10.3168/jds.2010-4096.
[65] L.K. Jr, P. Williams, R.J. Schmidt, W. Hu, A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows, J. Dairy Sci 91 (2008) 4793–4800, http://dx.doi.org/10.3168/jds.2008-1402.
[66] S.E. Hook, G. Wright, B.W. Mcbride, Methanogens: Methane Producers of the Rumen and Mitigation Strategies, 2010, 2010, pp. 50–60, http://dx.doi.org/10.1155/2010/945785.
[67] R. Bodas, N. Prieto, R. García-gonzález, S. Andrés, F.J. Giráldez, S. López, Manipulation of rumen fermentation and methane production with plant secondary metabolites ☆, Anim. Feed Sci. Technol 176 (2012) 78–93, http://dx.doi.org/10.1016/j.anifeedsci.2012.07.010.
[68] A.K. Patra, Z. Yu, Essential oils affect populations of some rumen bacteria in vitro as revealed by microarray (RumenBactArray) analysis, Front. Microbiol 6 (2015) 1–13, http://dx.doi.org/10.3389/fmicb.2015.00297.

[69] E.M. Ungerfeld, A Theoretical Comparison between Two Ruminal Electron Sinks, vol. 4, 2013, pp. 1–15, http://dx.doi.org/10.3389/fmicb.2013.00319.
[70] A.J. Flores, A.D. Garciarena, J.M. Hernández, K.A. Beauchemin, D. Colombatto, Effects of specific essential oil compounds on the ruminal environment, milk production and milk composition of lactating dairy cows at pasture, Anim. Feed Sci. Technol 186 (2013) 20–26, http://dx.doi.org/10.1016/j.anifeedsci.2013.09.001.
[71] R.S. Hegarty, J.P. Goopy, R.M. Herd, B. McCorkell, Cattle selected for lower residual feed intake have reduced daily methane production, J. Anim. Sci 85 (2007) 1479–1486, http://dx.doi.org/10.2527/jas.2006-236.
[72] D.J. Cottle, J.V. Nolan, S.G. Wiedemann, Ruminant enteric methane mitigation: a review, Anim. Prod. Sci 51 (2011) 491–514, http://dx.doi.org/10.1071/AN10163.
[73] W. Shi, C.D. Moon, S.C. Leahy, D. Kang, J. Froula, S. Kittelmann, C. Fan, S. Deutsch, D. Gagic, H. Seedorf, W.J. Kelly, R. Atua, C. Sang, P. Soni, D. Li, C.S. Pinares-Patiño, J.C. McEwan, P.H. Janssen, F. Chen, A. Visel, Z. Wang, G.T. Attwood, E.M. Rubin, Methane yield phenotypes linked to differential gene expression in the sheep rumen microbiome, Genome Res 24 (2014) 1517–1525, http://dx.doi.org/10.1101/gr.168245.113.
[74] R. Roehe, R.J. Dewhurst, C.A. Duthie, J.A. Rooke, N. McKain, D.W. Ross, J.J. Hyslop, A. Waterhouse, T.C. Freeman, M. Watson, R.J. Wallace, Bovine host genetic variation influences rumen microbial methane production with best selection criterion for low methane emitting and efficiently feed converting hosts based on metagenomic gene abundance, PLoS Genet 12 (2016) 1–20, http://dx.doi.org/10.1371/journal.pgen.1005846.