Review
Advances of Rumen Functional Bacteria and the Application of Micro-Encapsulation Fermentation Technology in Ruminants: A Review

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Abstract: Rumen functional bacteria are crucial for the homeostasis of rumen fermentation and micro-ecology. Cellulolytic bacteria, amylolytic bacteria, protein- and fat-degrading bacteria, lactic acid-producing bacteria, lactic acid-consuming bacteria, methanogens, and others can all be found in the rumen flora and help the host and other microorganisms convert feed into energy. For instance, Ruminococcus flavefaciens, Ruminococcus albus, and Fibrobacter succinogenes are the three most prevalent fiber-degrading bacteria. The digestion and metabolism of various nutrients and the absorption in rumen epithelium can greatly enhance host defense mechanisms and health production in ruminants. However, directly feeding live bacteria is prone to negative environmental effects. Therefore, the micro-encapsulation of film-forming and acid-resistant wall materials can become a great means of encapsulating naked bacteria into tiny particles. It can maintain the activity of functional flora, boost the function of the intestinal barrier, and improve its capacity for colonization on the surface of the rumen and colon mucosa. Therefore, the present review evaluates the latent progress of main functional bacteria and the applied techniques of micro-encapsulation in the rumen, in order to provide more references for the development and application of rumen-functional bacteria.

Keywords: ruminant; rumen; functional bacteria; micro-encapsulation technology

1. Introduction

The rumen of ruminants has a large capacity as a natural feed fermenter that is rich in microbial structures (bacteria, anaerobic fungi, archaea, protozoa, and viruses). These microorganisms ferment and degrade nutrients to provide a significant quantity of energy and volatile fatty acids (VFA) for the host [1,2]. When animals are born, the digestive tract does not contain any microorganisms, as direct contact with the mother is required to obtain rumen bacteria. Within 24 h of birth, facultative anaerobic bacterial groups start to develop on the rumen wall [3–5]. The rumen’s bacteria have the greatest diversity of all rumen microorganisms. More than 200 bacteria have been identified from the rumen, and the rumen fluid contains 10^9–10^11 bacteria/mL. Similar types of bacteria have different functions inside the rumen. Bacteroidetes, Firmicutes, and Proteobacteria are the three most prevalent phyla in rumen bacteria, and the dominant genus is unidentified Prevotella, Fibrobacter, Lachnospiraceae, Saccharofermentans, and Succinivibrio, according to Zhang et al. [6]. The relative quantification real-time PCR of the 16S rRNA gene in dairy-cow rumen fluid was used to study the bacterial diversity and discover Ruminococcus, Clostridium, Prevotella, Fibrobacter, Roseburia, and other relative abundances by Stevenson et al. [7]. Henderson et al. [8] analyzed 742 distinct rumen fluid samples from 35 different countries, representing 32 species. According to the findings, similar relative abundance ratios of Clostridium, Lachnospira, Ruminococcus, and Prevotella were found in the rumen, which may be referred to as the ‘rumen core flora’.
The rumen flora can be classified into cellulolytic bacteria, amylolytic bacteria, protein-degrading bacteria, fat-degrading bacteria, lactic acid-producing bacteria, lactic acid-utilizing bacteria, and methanogens, etc., all of which can facilitate the conversion of feed into energy for the host and other microorganisms [9,10]. In addition to regulating the gastrointestinal environment, the complex interplay of habituation, competition, and symbiosis established by the interaction of functional flora also affects the host’s physiological processes through their metabolites. An adequate supplement of functional flora is necessary to change the microbial population in the rumen or hindgut, change its fermentation mode, restore the effect of dominant flora, inhibit the secretion of toxins by harmful bacteria, improve disease resistance, and increase production performance [11–13].

The direct feeding of naked bacteria is prone to environmental influences and will alter the composition of gastrointestinal tract microbiota, resulting in gastrointestinal integrity and immune response [14] as the pH value of the rumen is very different from the acidic environment of the stomach and small intestine. For instance, it might affect weaned lambs’ feed intake, digestibility, and growth performance [15]. Later, people have employed the micro-encapsulation technique to protect active ingredients, coating the flora with a film-forming, acid-resistant wall material to create tiny particles that were then administered to animals. The package is protected from the outside environment to increase the internal environmental resistance [16]. With the help of this technology, it is possible to preserve the bacterial flora, regulate when and where it is released, and increase its survival rate. To give additional context to the commercial use of micro-encapsulating ruminant flora, this article will discuss the scientific advancement of this technology in the micro-encapsulation of rumen flora, its mechanism of action, and its application effects.

2. General Situation of Rumen Functional Bacteria

Plant cell walls are broken down by bacteria, protozoa, and fungi in the rumen of ruminants. Rumen bacteria dominate cellulose digestion because of their superiority in numbers and their variety of metabolic pathways, regardless of the intricate interactions between microorganisms in the entire rumen ecosystem. About 50% of the crude fiber consumed by ruminants is digested in the rumen, where rumen fiber-degrading bacteria play a significant role in the breakdown of cellulose or hemicellulose in the diet [17]. Among the fiber-degrading bacteria, the three most common types include Ruminococcus flavefaciens, Ruminococcus albus, and Fibrobacter succinogenes [18]. Nutrients such as starch, xylan, and pectin can be broken down by amylolytic bacteria, Prevotella ruminicola, and Streptococcus bovis [19]. Additionally, some bacteria, such as Fibrobacter succinogenes and Butyrivibrio fibrisolvens, can break down both cellulose and starch [20].

Protein-degrading bacteria, primarily Ruminobacter amylophilus and Butyrivibrio fibrisolvens, transform plant proteins and non-protein nitrogen, which the host body cannot utilize, into flora microbial proteins for their usage [21]. Numerous other bacterial species, including Clostridium spp., Eubacterium ruminantium, Prevotella spp., Streptococcus bovis, Bacteroides ruminicola, Fibrobacterium sp., and Selenomonas ruminantium, have the potential to degrade proteins in specific ways. Rumen soluble protein degradation is primarily regulated by R. amylophilus, B. fibrisolvens, Prevotella spp., and S. bovis. These organisms can also alter the pace of soluble protein degradation and result in nitrogen loss from the rumen in the form of ammonia [22]. The only bacterium in the rumen that can break down fat is Anaerovibrio lipolytic, which is primarily employed to break down fat and consume lactic acid [23].

Many bacteria can make lactic acid, an essential intermediate product in the rumen, but several experts agree that Lactobacillus, Streptococcus, Enterococcus, and Pediococcus are the principal lactic acid-producing bacteria. Numerous strains of bacteria from these genera can be utilized as probiotics and are well known for regulating the host’s digestive system, immunological system, and digestibility. Ruminal acidosis can be brought on by an excessive buildup of lactic acid in the rumen as a result of the dysbiosis between lactic acid-producing bacteria, and lactic acid-utilizing bacteria [24,25]. Methanogens, which
are primarily found in the rumen and lower intestine, can convert carbon and energy sources into methane by using the reducing equivalents created by rumen fermentation [26]. Methane warms the planet 21 times more effectively than carbon dioxide. Methane is a byproduct of anaerobic fermentation in the rumen and is produced by methanogens. Ruminant intestinal methane emissions can be decreased by adding organic and inorganic feed additives [27]. According to the primary nutrients utilized, the rumen functional bacteria can be classified into seven different types, as shown in Table 1.

Table 1. Classification of major rumen functional bacteria.

| Classification                  | Latin Name of Bacteria | Gram Staining | Function                                                                 | Source                  |
|---------------------------------|------------------------|---------------|--------------------------------------------------------------------------|-------------------------|
| Cellulolytic bacteria           | *Ruminococcus flavefaciens* | G+            | degrade cellulose, hemicellulase, xylan                                 | Yeoman et al. [18]      |
|                                 | *Ruminococcus albus*    | G+            | degrade cellulose, hemicellulase, xylan                                 |                         |
|                                 | *Fibrobacter succinogenes* | G−            | ferment cellulose and cellobiose utilize cellulose, starch, and other polysaccharides, secrete pectinase, utilize xylan | Rodríguez Hernández et al. [28] |
|                                 | *Butyrivibrio fibrisolvens* | G−            |                                                                         |                         |
| Amylolytic bacteria             | *Streptococcus bovis*   | G+            | degrade starch to produce lactic acid                                    | Cerqueira et al. [19]   |
|                                 | *Prevotella ruminicola*  | G−            | degrade starch, xylan, pectin fermentation, xylan                      | Anderson et al. [29]    |
|                                 | *Ruminobacter amylophilus* | G−            | ferment starch, degrade protein utilize cellulose, starch and other polysaccharides, secrete pectinase, utilize xylan | Anderson et al. [29]    |
|                                 | *Butyrivibrio fibrisolvens* | G−            |                                                                         | Cotta et al. [30]       |
| Protein-degrading bacteria      | *Ruminobacter amylophilus* | G−            | ferment starch, degrade protein                                        |                         |
|                                 | *Butyrivibrio fibrisolvens* | G−            |                                                                         |                         |
| Fat-degrading bacteria          | *Anaerovibrio lipolytica* | G−            | utilize fat and lactic acid                                             | Prins et al. [31]       |
| Lactic acid-producing bacteria  | *Bifidobacterium lactis* | G+            | produce acetic acid, lactic acid, inhibit spoilage bacteria             | Uusitupa et al. [32]    |
|                                 | *Lactobacillus acidophilus* | G+            | produce lactic acid and acetic acid                                    | Anjum et al. [33]       |
|                                 | *Streptococcus bovis*    | G+            | produce lactic acid and acetic acid                                    | Cerqueira et al. [19]   |
| Lactic-acid-utilising bacteria  | *Selenomonas ruminantium* | G−            | utilize lactic acid to produce acetic and propionic acids               | Fan et al. [34]         |
|                                 | *Megasphaera elsdenii*   | G−            | ferment fructose, lactic acid                                          | Monteiro et al., Chen et al. [25,35] |
| Methanogens                     | *Methanobrevibacter ruminantium* | G+            | reduce CO2, CH4                                                        | Ma et al. [36]          |
|                                 | *Methanomicrobium mobile* | G−            | reduce CO2, CH4                                                        | Yanagita et al. [37]    |

3. Rumen Functional Bacteria Development Technology and Its Corresponding Effects

A potential strategy to enhance the gut microbiota and avoid disease is to feed the functioning flora. Feeding the flora can enhance the organism’s growth and development as well as prevent and treat diseases without running the risk of infecting the host or its animal products. This has minimal negative side effects and significant positive economic benefits [38–40]. It can also provide the technical resources needed for breeding without the use of antibiotics [41,42]. An appropriate flora system in the gastrointestinal tract can be established and kept in check by including a well-defined functional flora that can colonize in animals.

Feeding pre-weaning lambs a starter enriched with functional flora can change the makeup of the rumen epithelial bacterial population and the expression of a few key immune-related genes, all of which are advantageous for weaning lambs [43]. By examining the microbiota–gut–brain axis, Ban et al. [44] discovered that the metabolites of the intestinal microbiota can have an impact on the host neurons and endocrine system as well as controlling the release of particular immune mediators. It has been established that gut bacterial colonization is one of the essential elements for the development of the gut and nervous systems. Wiley et al. [45] studied the bidirectional interaction of gut microbiota with the enteric nervous system and the central nervous system using germ-free animals. Immunity, the neuroendocrine system, and the vagus nerve are the three main channels of communication between the brain and the gut. By means of various biologically active substances, these three pathways enable a two-way information exchange between the brain and the gut, where the gut flora is also an essential component of the body. Through these three pathways of the gut–brain axis, the gut microbiota not only affect the gastrointestinal
system but also form the gut microbiota–gut–brain axis, which affects brain function and behavior. The host’s immunological responses, metabolic functions, and neuroendocrine pathways are all impacted by functioning microbiota [46]. Specific combinations of dominant microbial communities may be generated through early intervention in the rumen microbiota of young animals [47], which may have a huge potential for enhancing function and health.

Six local sheep were split into two groups in an experiment by Herdian et al. [48]. After treatment with a basal diet and probiotic and organic mineral complex separately, it was discovered that adding *Lactobacillus* and organic minerals to the meal improved meat quality, and lowered cholesterol levels. In order to feed neonate lambs, Ishaq et al. [49] isolated five fibrolytic bacteria from the rumen of North American moose (*Alces alces*). After nine weeks, they saw an increase in rumen bacterial diversity, no improvement in body weight or wool quality, but a modest improvement in daily grain efficiency. Gkouakis et al. [50] found that *Lactobacillus plantarum* PCA 236 can usefully regulate goat fecal microbiota and milk fatty-acid composition. The application impact and mechanism of rumen functional bacteria have been extensively studied by both domestic and international specialists and academicians, but their commercial development and utilization technology still need to be further explored.

4. Micro-Encapsulation Technology of Micro-Organisms

Small-scale packaging innovation is micro-encapsulation technology. There have been more than 200 different ways to prepare micro-encapsules since they were first created by Wuster and Green in the 1930s. By using micro-encapsulation technology, a solid, liquid, or gas can be enclosed within the micro-encapsule wall. Micro-encapsules come in a variety of morphologies, such as spherical, kidney-shaped, grain-shaped, and block-shaped. They are frequently utilized in food, medicine, and other products. In pharmaceutical applications, the dosage of the medicine is decreased and the duration of the drug’s effectiveness is extended, allowing for targeted drug release; in dietary applications, the odor of some raw materials can be concealed [51,52]. By using micro-encapsulation technology, it is possible to isolate the rumen’s microorganisms from their surroundings and reduce the impact of gastric acid, bile, enzymes, and other chemicals [53,54].

The concepts of micro-encapsulation can be categorized into the chemical process, and physical process due to the various wall and core materials [55]. The physical process creates micro-encapsules by utilizing physical and mechanical principles. Extrusion, emulsification, spray drying, and other physical procedures are currently the more advanced and commercialized techniques. The extrusion method [56–58] evenly distributes the core materials into the carbohydrates (wall materials), and then extrudes the mixture of the core material and the wall material into the cooling medium under pressure. This process quickly dehydrates and cools down, causing the wall material to precipitate and harden to form micro-encapsules. The core material and the wall material are dissolved in the solvent during the spray drying method [37], and the resulting mixture is atomized before being heated in the heating chamber as tiny droplets. The solvent evaporates during the heating process, and after separation, the micro-encapsulated particles are obtained. The size of the micro-encapsules can be varied, controlled, and adjusted using the emulsification method [59]. Spray drying is used to create rumen bypass microcapsules, which stop microbial hydrogenation processes (neutral pH) in the rumen. The porous starch is used as the base material for the microcapsules, and the triple coating process is used. Microcapsules are very stable in neutral solutions that closely resemble the pH of the rumen. Furthermore, only about 85% of microcapsules are effectively released within 30 min, and about 65% of them are resistant to digestion in rumen fluid [60]. A homogeneous and stable water-in-oil (W/O) emulsification is created by first thoroughly combining the core material and the wall material, adding the vegetable oil, and stirring at a high speed for emulsification. The core material is embedded in the micro-encapsules of the film generated by the wall material and the curing agent when the droplets are introduced, causing the wall material
solution to react with it and solidify. The fundamental idea behind the chemical process is as follows: tiny monomers or macromolecules are polymerized in a solution to create the polymer film-forming material, which is then coated onto the capsule’s core to create micro-capsules [61,62]. The physical and chemical process involves altering certain variables, such as pH, temperature, or the addition of electrolytes, to deposit the film-forming material walls in solution and coat the cores to produce micro-capsules [63–65]. New micro-encapsulation technologies are continuously being generated and developed as a result of the expansion of application domains and the advancement of micro-capsule research. However, there are extra requirements on the procedure, cost, efficiency, load, and output because the core material must be kept physiologically active at all times during the preparation process. The preparation process has evolved technologically from a single physical procedure to a chemical method or a combination of chemical methods. In order to assure their biological safety, the choice of wall materials must also have a particular level of mechanical strength, solubility, fluidity, falsifiability, permeability, stability, and economy [66].

Whether natural or manufactured, many organic and inorganic polymer materials can be used as wall materials. It has been demonstrated that organic materials such as polylactic acid, glycolic acid copolymer, polycaprolactone, polypeptide, starch, gelatin, dextran, albumin, and polylactic acid have the benefits of simple operation, high encapsulation efficiency, and low toxicity. Additionally, they have extended half-lives and are challenging to change in terms of their biochemical features [67,68]. Good chemical characteristics and thermal stability are characteristics of inorganic materials, including double metal hydroxides, calcium carbonates, phosphates, silicates, and clays [69]. Table 2 describes the primary micro-encapsulation methods in detail.

Table 2. Principles, methods, wall materials, advantages and disadvantages of micro-encapsulation technology.

| Principles          | Methods                  | Wall Materials                          | Advantages                                      | Disadvantages                                      | Source                           |
|---------------------|--------------------------|-----------------------------------------|-------------------------------------------------|----------------------------------------------------|----------------------------------|
| Physical process    | Spray drying             | phthalate, (modified) starch, soy protein isolates, etc. | low cost, simple process, convenient transportation and storage | uneven particle size, low embedding rate           | Yanagita et al. [37]            |
|                     | Extrusion               | alginate, calcium chloride, gellan gum, protein, etc. | good sealing, suitable temperature, long storage period | low production efficiency | Lee et al., Kailasapathy et al., Yao et al. [56–58] |
|                     | Emulsification          | gum arabic, gelatin, chitosan, etc. | strong stability, suitable temperature          | low production efficiency equipment requirements are high, and sieving is required after granulation easily damaged, low production efficiency, and many influencing factors | Ji et al. [59]                  |
|                     | Freeze drying           | maltodextrins, sorbitol, gums, trehalose, etc. | core material damage is small                   |                                                    | Fonseca et al. [70]             |
|                     | Fluid bed coating       | casein, alginate, waxes etc.            | uniform particle size                           |                                                    | Knezevic et al. [71]            |
|                     | Electrospinning and     | pectin, guar gum, cellulose, chitosan, alginate etc. | nanoscale, low cost, high voltage, complex equipment |                                                    | Dierings de Souza et al. [72]   |
| Chemical process    | Interfacial Polymerization | polylamide, polyurea, polyester, polyurethane, etc. | good sealing, low cost and simple process       | part of the monomer remains in the micro-encapsules | Mytara et al. [61]              |
|                     | In situ polymerization  | methacrylate, polystyrene, urea-formaldehyde resin, polyurethane, etc. | easy to form spherical shape, wider application | some monomers remain in the micro-encapsules, the process is more complicated | Jeoung et al. [62]              |
### Table 2. Cont.

| Principles               | Methods          | Wall Materials                  | Advantages                                                                 | Disadvantages                                                                 | Source                        |
|--------------------------|------------------|---------------------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------------|-------------------------------|
| Physico and chemical process |                  |                                 |                                                                              |                                                                                |                               |
| Complex coacervation      | gelatin, gum arabic, etc. | high temperature resistance, high yield and low loss of biological activity | reaction conditions and costs are difficult to control, and storage period is short | Hernández-Nava et al. [63]                                                    |                               |
| Self-coacervation         | agar, sodium alginate, chitosan etc. | high temperature resistance, high productivity | high cost and complex process                                                  | Jing et al. [64]                                                            |                               |
| Phase separation          | ethyl cellulose, polyethylene, polystyrene, nitrocellulose, etc. | the process is simpler           | time consuming and risk of contamination                                      | Abulateefeh et al. [65]                                                      |                               |
| Supercritical CO₂ Method  | gum arabic, sodium alginate, chitosan etc. | high production efficiency, low investment | unstable shape, low load                                                      | Chen et al. [73]                                                            |                               |
| Layer by Layer method     | sodium alginate, chitosan, pectin, gum arabic, etc. | controlled release, high stability, nanoscale | complex process                                                              | Tong et al. [74]                                                            |                               |

5. Application of Microbial Micro-Encapsulation Technology

An efficient micro-encapsule should retain its original qualities and performance while in use, minimize the impact of the gastrointestinal tract’s acid-base environment, and maintain the stability of its core material [75]. It can attach to and populate the colon’s inner wall once it reaches the colon. Inclusions are being released on time [76,77]. Due to various types of micro-encapsule walls and core materials, the core material’s release dynamics primarily depend on its solubility in the medium, the solvent’s capacity for diffusion, the swelling of the polymer, the decomposition of the wall material, and external factors, such as the effects of touch, light, pH, etc. The five types of micro-encapsule release mechanisms include diffusion, dissolution, erosion, osmosis, and rupture [68]. The danger of cross-contamination during the mixing or storage of wall components and core materials should be taken into consideration during actual production [78].

*Lactobacillus* and *Bacillus subtilis* were enclosed in various micro-encapsule compartments by Zhao et al. [16], using sodium alginate, methylcellulose, and fiber nanocrystals as the wall materials for the bilayer micro-encapsules. In simulated gastric juice, the activity of the naked bacteria and micro-encapsules was assessed at 0, 15, 30, and 60 min. According to the findings, 70% of the activity was still going strong 60 min after embedding. Additionally, the outcomes of the quantitative analysis and inflammation slices demonstrated that feeding high-fat-induced mice with bilayer micro-encapsules can enhance the imperfect morphology of intestinal villi, reduce intestinal permeability, and restore barrier proteins, which are beneficial for the treatment of the metabolic syndrome. Chang et al. [79] developed a *Lactobacillus acidophilus* micro-encapsule for feeding ruminants using electrostatic spinning. All micro-encapsule groups showed no discernible change after being placed in the intestinal fluid and rumen fluid for 16 h and 48 h, respectively. In contrast to the naked bacteria group, where there was no significant change, *L. acidophilus*’ relative abundance in intestinal fluid considerably increased in the micro-encapsule group. Micro-encapsules of *Lactobacillus delbrueckii*, which have a good rate of micro-encapsulation, gastrointestinal tolerance, and storage stability, can help control the disruption of the intestinal flora brought on by a high-fat diet. Whole-fat goat’s milk and/or prebiotics (inulin and/or oligofructose) were used as carriers to micro-encapsulate *Bifidobacterium* BB-12. Micro-capsules generated using solely whole-fat goat’s milk exhibited the best survival rate (9.58 log CFU.g⁻¹) and encapsulation efficiency (97.43%) under in vitro simulations of the gastrointestinal tract after heat treatment. Micro-capsules made with whole-fat goat’s milk performed the best after being exposed to in vitro simulated gastrointestinal conditions (94.29%), followed by micro-capsules made with whole-fat goat’s milk and inulin (86.77%). Following heat treatment of the micro-capsules, all carrier agents increased the survival rate of *Bifidobacterium* BB-12 [80]. Five different bacterial species were tested by Piano et al. [81]: *Lactobacillus acidophilus* LA02 (DSM 21717); *Lactobacillus rhamnosus* LR04 (DSM 16605);
L. rhamnosus GG, or LGG (ATCC 53103); L. rhamnosus LR06 (DSM 21981); and Bifidobacterium lactis BS01 (LMG P-21384), which were all embedded and compared to naked bacteria. Micro-encapsulated bacteria were delivered for 21 days at $1 \times 10^9$ cfu/strain/d (total $5 \times 10^9$ cfu/d), while uncoated strains were given at $5 \times 10^9$ cfu/strain/d (total $25 \times 10^9$ cfu/d). The findings showed that every strain can colonize the intestines, but the micro-encapsulated strains have a colonization capacity that is five times greater than that of naked bacteria. This can enhance the disease’s immune-regulatory potential.

6. The Limitations of Micro-Encapsulation Technology and the Application of Rumen Functional Bacteria

As stated above, there are few studies on the use of rumen functional bacteria encapsulation in ruminant animal models, and the majority of the technical research on microbial micro-encapsulation now focuses on probiotics and non-ruminant animal models. It is necessary to increase efforts to excavate various wall materials to prepare functional bacteria micro-encapsule products and explain their application through a large number of experiments.

Moreover, it is necessary to increase efforts to pass and embed relevant strains to avoid rumen degradation to reach the intestinal tract effect. Additionally, there are restrictions on the micro-encapsulation preparation method, embedding volume, stability, and metabolite expression. External conditions include too much oxygen, high relative humidity and temperature, too much pressure, and too much heat produced by mechanical stirring during the preparation process, which will render functional microorganisms inactive. One or more layers of polymer molecular structures may be coated on the surface of the microcapsules to enhance production performance with zein based on Bifidobacterium bacteria embedded in alginate, for example, which can significantly increase survival rates [67]. Streptococcus thermophilus (IFFI 6038) cells were combined with trehalose and alginate by extrusion to create the micro-encapsules. To create chitosan-trehalose-alginate micro-encapsules with a shell matrix structure, these capsules were then coated with chitosan. An ideal balance of stability and acid resistance is demonstrated by this glycan-trehalose-alginate micro-encapsule structure [82].

However, many thin-walled materials are limited because larger micro-encapsules readily dissolve in the rumen. Additionally, there are no assays for stability and activity following micro-encapsulation or in-depth studies of each process, and the processes from encapsulation storage to colonization in the hindgut are independent. When more than a specific number of functional bacteria are consumed simultaneously, their metabolites may potentially impact the overall expression of the host genes [62]. Therefore, before creating micro-encapsules, several factors such as safety, functionality, and technological quality need to be taken into account. The safety element of micro-encapsules should be first considered to cover things such as antibiotic resistance and whether or not they were produced from the gastrointestinal systems of healthy animals or from some beneficial fermented products [82,83]. Gastrointestinal motility and persistence, immunomodulatory, antagonistic, and antimutagenic characteristics are functional features, which must also be able to be produced in an industrial setting. They also need to keep their functionality while being stored and in the feed to which they are introduced, without emitting off-tastes [84].

All in all, further studies are still required to enhance or improve the current technology to overcome the limits of the micro-encapsulation technology of these rumen functional bacteria in the future.

7. Conclusions

In conclusion, the benefits of preparation and micro-encapsulation technology have gradually emerged as a new technique in probiotic preparation research. A range of technologies is urgently required to ameliorate the current situation (e.g., inadequate rumen functional bacteria resources and poor coating technology). One such technology is the regulation of microbes, which is highly significant. The benefits of preparation and
micro-encapsulation technology mean that they have gradually emerged as new techniques in research. For the micro-encapsulation technology of rumen functional bacteria, a more in-depth study is needed on the choice of wall materials, the manufacturing process, cost control, and maintenance of safety.

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