

ENCAPSULATION FORMULATIONS FOR ANAEROBIC BACTERIA, COMPOSITIONS, AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

- [0001] The present application claims the priority benefit of U.S. Provisional Patent Application No. 63/597,285, filed November 8, 2023, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

Field of Disclosure

- [0002] The present disclosure relates to encapsulation formulations, compositions, and uses thereof for anaerobic bacteria.

Background

- [0003] *Megasphaera elsdenii* (i.e., *M. elsdenii*) is an anaerobic, non-motile, and gram-negative diplococci that utilizes lactate as a preferred carbon source and can help to prevent acidosis, which is a common digestive disorder that affects millions of beef and dairy cattle each year.
- [0004] When cattle and other ruminants ingest large quantities of starchy foods (e.g., cereal grains) or simple sugars, opportunistic microorganisms in the stomach can rapidly ferment these compounds into lactic acid. Lactic acid is a potent organic acid and can lead to lactic acidosis, which can disrupt normal digestive activity and cause extensive damage to the digestive tract lining in ruminants. Affected animals have suboptimal performance. And, the most acute form of lactic acidosis can cause irreversible damage to an animal's digestive and respiratory systems, as well as increased mortality rates.
- [0005] *M. elsdenii* can help to control lactic acidosis by converting lactic acid into volatile fatty acids (VFA; e.g. butyrate, propionate, and acetate), which are beneficial for the animal. The volatile fatty acids are further metabolized by the animal and other bacteria. However, *M. elsdenii* populations in the gastrointestinal tract (GIT) of ruminants are often at levels too low to prevent the risk of acidosis. Accordingly, a liquid culture of

live cells from a strain of *M. elsdenii*, Lactipro[®], was developed to increase the rate of colonization of *M. elsdenii* in the gastrointestinal tract of ruminants. *See*, e.g., U.S. Patent No. 7,550,139. However, there are practical constraints that have limited the use of products containing *M. elsdenii*, including the difficulty of maintaining *M. elsdenii* products under the anaerobic conditions required by the organism and the difficulty of transporting the *M. elsdenii* products from production facility to site of end-use within 14 days, after which time the viability of *M. elsdenii* in the product decreases significantly. A product containing freeze-dried *M. elsdenii* (Lactipro NXT[®] and Lactipro FLX[®]) has a longer shelf life than Lactipro[®], but there is a desire for further improved compositions, which are encapsulated and can be added in feed, feed additives, or on its own. *See* WO 2018/144653 A1, which is herein incorporated by reference in its entirety. These compositions will allow for further use in the animal health market.

[0006] Cattle are at risk for acidosis when they are not adequately adapted to a high starch diet, or when they go off feed for any reason at all, such as weaning, transportation, sickness/injury, muddy pens, extreme heat or cold, storms, equipment break down, or processing. Lactic acid accumulation in the rumen can, in those cases, result in acidosis with negative impacts for the animal (i.e. low feed intake, poor performance, and even death). Therefore, minimizing acidosis is important for producers, especially during diet adaptation when it is most prevalent. Traditionally, ruminants in feedlots are adapted gradually over 3-4 weeks from a high forage to a high concentrate diet. A gradual increase of a high concentrate diet minimizes the accumulation of lactate in the rumen. *M. elsdenii* has the potential to mitigate acidosis and shorten the transition period of feedlot cattle from a high-forage diet to a high concentrate diet by providing an active population of *M. elsdenii* to limit lactic acid accumulation. *M. elsdenii* is a strict anaerobic bacteria, and thus far, commercialized as a drench (LactiproNXT[®]) or bolus (LactiproFLX[®]), with a limited window for administration when the animals are handled or processed. This makes it unsuitable for daily administration or impromptu administration as required for off feed events, such as weather events and/or equipment break down.

[0007] Similarly, *M. elsdenii* has been considered an option for decreasing the occurrence of metabolic conditions, such as subacute ruminal acidosis (SARA), during the transition period for dairy cow from the dry period to lactation. SARA can have severe implications in dairy cows such as decreased milk production and laminitis, the later is often

associated with lameness and can result in premature culling. Previous studies have shown the importance of timely administration of *M. elsdenii*. Use of a drench or bolus with a limited window of administration prevent producers (e.g., dairy cows) from receiving the full benefit of *M. elsdenii*.

[0008] Therefore, there is a need for the development of stable *M. elsdenii* formulations that could withstand storage and mixing in feed while still releasing the bacteria in the rumen of cattle. This is key for commercial feedlot and dairy cattle, which would allow for a daily or a more targeted administration. There is also a need for stable formulations of other anaerobic bacteria that can release in the rumen, including *Bifidobacterium*, such as *B. breve*, *Lactobacillus*, such as *L. plantarum*, *Bifidobacterium*, such as *B. animalis* subsp. *lactis*, *Pediococcus*, such as *P. acidilactici*, *Lactobacillus*, such as *L. casei*, *Fibrobacter*, such as *F. succinogenes*, *Butyrivibrio*, such as *B. fibrisolvens*, and *Ruminococcus*, such as *R. flavefaciens* *Blautia*, such as *B. obeum*, *Clostridium*, such as *C. butyricum*, *Akkermansia*, such as *A. muciniphila* as well compositions and uses thereof.

BRIEF SUMMARY OF THE DISCLOSURE

[0009] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells are viable in the composition.

[0010] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a feed or feed additive.

[0011] In some aspects, the *Megasphaera elsdenii* cells in the composition are viable for up to 48 hours at 25° C and pH 7.0 after being added to the feed or feed additive.

[0012] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is

coated by the layer of one or more lipids. In some aspects, administration of the composition to a ruminant results in viable bacterial cells being released in the rumen.

[0013] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a temperature of at least 40° C to 60° C at pH 7.0 for 4-18 hours.

[0014] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a pH between 3-7 at 25° C for up to 48 hours.

[0015] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a pH between 3-7 and a temperature between 25° C to 60° C for up to 48 hours.

[0016] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being processed through a micromachine system.

[0017] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for up to 14 hours.

- [0018] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for up to 48 hours.
- [0019] In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for about 14 hours to about 48 hours.
- [0020] In some aspects, the particle size of the *Megasphaera elsdenii* cells in the composition are less than about 1600 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells in the composition are less than about 400 μm .
- [0021] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are viable in the composition.
- [0022] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a feed or feed additive.
- [0023] In some aspects, the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable for up to 48 hours at 25° C and pH 7.0 after being added to the feed or feed additive.
- [0024] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In

some aspects, the administration of the composition to a ruminant results in viable bacterial cells being released in the rumen.

[0025] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a temperature of 40° C to 60° C at pH 7.0 for 4-18 hours.

[0026] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a pH between 3-7 at 25° C for up to 48 hours.

[0027] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a pH between 3-7 and a temperature between 25° C to 60° C for up to 48 hours.

[0028] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a micromachine.

[0029] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one

or more lipids, wherein the core is coated by the layer of one or more lipids, In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine for up to 14 hours.

[0030] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine for up to about 48 hours.

[0031] In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine for about 14 hours to about 48 hours.

[0032] In some aspects, the particle size of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state cells in the composition are less than about 1600 μm . In some aspects, the particle size of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state cells in the composition are less than about 400 μm .

[0033] In some aspects, at least 15%, 20%, 30%, 40%, 50%, 60%, about 70%, 80%, 90%, or 99% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are viable in the composition.

[0034] In some aspects, the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are dried.

[0035] In some aspects, the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are dried by spray drying, electrospray drying, vacuum drying, jet drying, freeze-drying, or combinations thereof.

[0036] In some aspects, the composition comprises from about 0.1% to about 15% (w/w) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state.

[0037] In some aspects, the composition comprises anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state from about 1×10^4 to about 1×10^{10} CFU/g.

[0038] In some aspects, the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are selected from the group consisting of *Bifidobacterium breve*,

Lactobacillus plantarum, *Bifidobacterium animalis subsp. lactis*, *Pediococcus acidilactici*, *Lactobacillus casei*, *Megasphaera elsdenii*, *Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, *Blautia obeum*, *Clostridium butyricum*, *Akkermansia muciniphila* and combinations thereof.

- [0039] In some aspects, the composition is a granule, capsule, minicapsule, microcapsule, tablet, minitab, or microtablet.
- [0040] In some aspects, the composition has a moisture content of about 5% (w/w) or less.
- [0041] In some aspects, the one or more lipids in the core is selected from the group consisting of animal oils or fats, vegetable oils or fats, triglycerides, free fatty acids, animal waxes, beeswax, lanolin, shell wax, Chinese insect wax, vegetable waxes, carnauba wax, candelilla wax, bayberry wax, sugarcane wax, mineral waxes, synthetic waxes, natural and synthetic resins, and mixtures thereof.
- [0042] In some aspects, the one or more lipids in the core is an animal fat or oil and/or a plant fat or oil.
- [0043] In some aspects, the plant fat or oil is selected from the group consisting of canola oil, cottonseed oil, hydrogenated cottonseed oil, peanut oil, corn oil, olive oil, soybean oil, hydrogenated soybean oil, sunflower oil, safflower oil, coconut oil, palm oil, hydrogenated palm oil, linseed oil, tung oil, castor oil and rapeseed oil.
- [0044] In some aspects, the plant fat or oil is hydrogenated palm oil.
- [0045] In some aspects, the free fatty acid is myristic acid, lauric acid, or stearic acid, or combinations thereof.
- [0046] In some aspects, the one or more lipids in the core has a melting point of about 40° C to about 85° C.
- [0047] In some aspects, the one or more lipids in the core has a melting point of about 55° C to about 75° C.
- [0048] In some aspects, the one or more lipids coating the core is selected from the group consisting of animal oils or fats, vegetable oils or fats, triglycerides, free fatty acids, animal waxes, beeswax, lanolin, shell wax, Chinese insect wax, vegetable waxes, carnauba wax, candelilla wax, bayberry wax, sugarcane wax, mineral waxes, synthetic waxes, natural and synthetic resins, and mixtures thereof.

- [0049] In some aspects, the one or more lipids coating the core is an animal fat or oil and/or a plant fat or oil.
- [0050] In some aspects, the plant fat or oil is selected from the group consisting of cottonseed oil, hydrogenated cottonseed oil, peanut oil, corn oil, olive oil, soybean oil, hydrogenated soybean oil, sunflower oil, safflower oil, coconut oil, palm oil, hydrogenated palm oil, linseed oil, tung oil, castor oil and rapeseed oil.
- [0051] In some aspects, the plant fat or oil is hydrogenated palm oil or hydrogenated cottonseed oil.
- [0052] In some aspects, the free fatty acid is myristic acid, lauric acid, or stearic acid.
- [0053] In some aspects, the one or more lipids coating the core has a melting point of about 55° C to about 80° C.
- [0054] In some aspects, the one or more lipids coating the core has a melting point of about 55° C to about 75° C.
- [0055] In some aspects, the at least one carrier comprises maltodextrin, sucrose, starch, cellulose, clay, biochar, lignin-derivatives, sugar alcohols, or combinations thereof.
- [0056] In some aspects, the composition comprises a total of about 10% to about 99% (w/w) lipids.
- [0057] In some aspects, the composition comprises a total of about 70% to about 80% (w/w) lipids.
- [0058] In some aspects, the composition comprises from about 10% to about 30% (w/w) of at least one carrier.
- [0059] In some aspects, the composition comprises from about 15% to about 25% (w/w) of at least one carrier.
- [0060] In some aspects, the composition is from about 0.1 mm to about 3 mm diameter in size.
- [0061] In some aspects, the composition is from about 0.2 mm to about 0.6 mm diameter in size.
- [0062] In some aspects, the composition is from about 0.2 mm to about 0.4 mm diameter in size.
- [0063] In some aspects, the core comprises from about 1% to about 99% of the composition.

- [0064] In some aspects, the core comprises from about 10% to about 90% of the composition.
- [0065] In some aspects, the core comprises from about 25% to about 80% of the composition.
- [0066] In some aspects, the one or more lipids coating the core comprises from about 1% to about 99% of the composition.
- [0067] In some aspects, the one or more lipids coating the core comprises from about 5% to about 75% of the composition.
- [0068] In some aspects, the composition has a density from about 0.6 g/mL to about 1.2 g/mL.
- [0069] In some aspects, the composition has a porosity from about 10% to about 60%.
- [0070] In some aspects, at least 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99% of the *Megasphaera elsdenii* cells are viable in the composition.
- [0071] In some aspects, the *Megasphaera elsdenii* cells are dried.
- [0072] In some aspects, the *Megasphaera elsdenii* cells are dried by spray drying, electrospray drying, vacuum drying, jet drying, freeze-drying, or combinations thereof.
- [0073] In some aspects, the composition comprises from about 0.1% to about 15% (w/w) *Megasphaera elsdenii* cells.
- [0074] In some aspects, the composition is from about 0.1 mm to about 1 mm diameter in size.
- [0075] In some aspects, the composition comprises *Megasphaera elsdenii* cells from about 1×10^4 to about 1×10^{10} CFU/g.
- [0076] In some aspects, the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state comprises from about 1×10^4 to about 1×10^{10} CFU/g in the composition.
- [0077] In some aspects, the composition further comprises one or more of antibiotics, antimicrobials, anti-coccidials, antiparasitics, sulfonamides, hormones, anti-bloat compounds, adrenergic receptor modulators, a phage, a prebiotic, probiotics, enzymes, essential oils, and/or a carbohydrate immune stimulant.
- [0078] In some aspects, provided herein is a feed additive composition comprising any of the compositions disclosed herein.
- [0079] In some aspects, the feed additive composition is a powder, granulate, particulate, pellet, cake, liquid, solid, suspension, emulsion, gel, or combinations thereof.

- [0080] In some aspects, provided herein is a feed comprising any of the compositions disclosed herein or any of the feed additive compositions disclosed herein.
- [0081] In some aspects, the feed further comprises an animal protein, a vegetable protein, corn, soybean meal, corn dried distillers grains with solubles (cDDGS), wheat, wheat proteins, gluten, wheat by products, wheat bran, wheat dried distillers grains with solubles (wDDGS), corn by products including corn gluten meal, barley, oat, rye, triticale, full fat soy, animal by-product meals, an alcohol-soluble protein, a zein, a maize zein, a kafirin, rice, paddy rice, extruded paddy rice, a protein from oil seeds, or a combination thereof.
- [0082] In some aspects, the animal protein or vegetable protein is selected from the group consisting of one or more of a gliadin or an immunogenic fragment of a gliadin, a beta-casein, a beta-lactoglobulin, glycinin, beta-conglycinin, cruciferin, napin, hordeins, keratins, feather or hair meals, collagen, whey protein, fish protein, fish meals, meat protein, egg protein, soy protein and grain protein.
- [0083] In some aspects, the protein from oil seeds is selected from the group consisting of soybean seed proteins, sun flower seed proteins, rapeseed proteins, canola seed proteins and combinations thereof.
- [0084] In some aspects, provided herein is a premix comprising a) any of the compositions disclosed herein, or any of the feed additive compositions disclosed herein; and b) at least one mineral and/or at least one vitamin.
- [0085] In some aspects, provided herein is a kit comprising a) i) any of the compositions provided herein; ii) any of the feed additive compositions provided herein; iii) any of the feeds provided herein; and/or iv) any of the premixes provided herein; and b) instructions for formulating and/or administering to a subject.
- [0086] In some aspects, provided herein is a method for treating or preventing a condition or disorder associated with lactic acid production in the gastrointestinal tract of a subject, comprising administering to the subject an effective amount of any of the feed additive compositions disclosed herein or any of the feeds disclosed herein.
- [0087] In some aspects, the condition or disorder is acidosis.
- [0088] In some aspects, the condition or disorder is ruminal acidosis.
- [0089] In some aspects, the condition or disorder is respiratory disease.
- [0090] In some aspects, the condition or disorder is laminitis.

- [0091] In some aspects, provided herein is a method for preventing or decreasing the growth of an opportunistic microorganism in the gastrointestinal tract of an animal, comprising administering to the animal an effective amount of any of the feed additive compositions disclosed herein or any of the feeds disclosed herein.
- [0092] In some aspects, the opportunistic microorganism is pathogenic.
- [0093] In some aspects, the opportunistic microorganism is *Salmonella*, *Escherichia Coli*, or *Campylobacter*.
- [0094] In some aspects, provided herein is a method for improving the growth performance of a subject comprising administering to the subject an effective amount any of the feed additive compositions disclosed herein or any of the feeds disclosed herein. In some aspects, improving the performance of a subject comprises of one or more of feed conversion ratio (FCR), weight gain, feed efficiency, carcass quality, reducing mortality, reducing morbidity, feed intake, average daily gain, carcass gain, bone mineralization, egg production, reduced digestive tract dysbiosis or dysfunction, milk composition (e.g., increased milk fat), and/or milk production compared to the performance of a subject that has not been administered the feed additive composition or feed.
- [0095] In some aspects, provided herein is a method for increasing starch digestibility, lowering fecal starch output, and/or preventing a decrease in the pH in the lower gastrointestinal tract in a subject comprising adding an effective amount of any of the feed additive compositions disclosed herein to a feed for administration to a subject, wherein the subject exhibits one or more of increased starch digestibility and/or lowered fecal starch output compared to a subject that has not been administered the feed additive composition.
- [0096] In some aspects, provided herein is a method for increasing operational efficiency of a farm, comprising administering to an animal on the farm an effective amount of any of the feed additive compositions disclosed herein or any of the feeds disclosed herein. In some aspects, the increased operational efficiency results decreased labor costs, decreased roughage transport costs, and decreased amount of roughage added to a feed or feed additive.
- [0097] In some aspects, the subject is a ruminant.
- [0098] In some aspects, the ruminant is selected from the group consisting of cattle, goats, sheep, giraffes, deer, gazelles, buffalo, reindeer, and antelopes.

- [0099] In some aspects, the cattle are beef cattle or dairy cattle.
- [0100] In some aspects, the subject is a non-ruminant.
- [0101] In some aspects, the non-ruminant is selected from the group consisting of: equine, poultry, and swine.
- [0102] In some aspects, the poultry is selected from the group consisting of: a chicken, a goose, a duck, a quail, a turkey, broiler, broiler breeder, a layer, or a pigeon.
- [0103] In some aspects, the poultry is a chicken.
- [0104] In some aspects, the feed additive composition or feed is provided to the subject for daily administration or for weekly administration.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

- [0105] **Figure 1 (FIG. 1)** shows *M. elsdenii* stability data for Potato Starch (PS), Wheat Starch (WS), and Corn Starch (CS) formulations stored in bulk at 4° C or -20° C. Formulation WS was not sampled after 4 months.
- [0106] **Figure 2 (FIG. 2)** shows *M. elsdenii* stability data (Log10 CFU/g of encapsulated *M. elsdenii* cells) over the course of 12 months of storage at 4° C.
- [0107] **Figure 3 (FIG. 3)** shows *M. elsdenii* recovery after passage, or not ("No micromachine"), through the bowl or continuous flow micromachine systems and 4 hours aerobic exposure to low pH and high moisture diet at room temperature.
- [0108] **Figure 4 (FIG. 4)** shows *in vitro* gas production (Ankom) using freeze dried culture, core, 8 (X1) formulation, 16 (X1) formulation, and vegetable oil formulation in semi-defined lactate media incubated at 39° C for 18 hours.
- [0109] **Figure 5 (FIG. 5)** shows *M. elsdenii* stability data for encapsulated product 8 (X1) stored in 1.5 g aliquots (10⁶ CFU) in Mylar pouch at -20° C and 4° C. Dashed line = extrapolated data.
- [0110] **Figure 6 (FIG. 6)** shows *M. elsdenii* recovery after mixing encapsulated product into a low pH/high moisture diet or a high pH/high moisture diet for up to 6 hours at 25° C or 52° C under aerobic conditions.
- [0111] **Figure 7 (FIG. 7)** shows *M. elsdenii* concentration in ground corn top dressed with freeze dried product and exposed to atmospheric conditions in the laboratory (inside) or outside in the sun. Exposure time * storage conditions interaction, P = 0.0007;

Exposure time effect, $P < 0.0001$; Storage conditions effect, $P < 0.0001$. Bars with a common superscript are not significantly different.

DETAILED DESCRIPTION OF THE DISCLOSURE

- [0112] The present disclosure relates to compositions comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells are viable in the composition.
- [0113] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a feed or feed additive.
- [0114] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, administration of the composition to a ruminant results in viable bacterial cells being released in the rumen.
- [0115] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a temperature of at least 40° C to 60° C at pH 7.0 for 4-18 hours.
- [0116] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a pH between 3-7 at 25° C for up to 48 hours.

- [0117] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a pH between 3-7 and a temperature between 25° C to 60° C for up to 48 hours.
- [0118] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are viable in the composition.
- [0119] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a feed or feed additive.
- [0120] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the administration of the composition to a ruminant results in viable bacterial cells being released in the rumen.
- [0121] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a temperature of 40° C to 60° C at pH 7.0 for 4-18 hours.

- [0122] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a pH between 3-7 at 25° C for up to 48 hours.
- [0123] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a pH between 3-7 and a temperature between 25° C to 60° C for up to 48 hours.
- [0124] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a micromachine.
- [0125] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine prior to addition to feedtruck for up to 14 hours.
- [0126] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a

vegetative state in the composition are viable after being added to a microbin of a micromachine prior to addition to feedtruck for up to 48 hours.

[0127] All publications, patents, and other references mentioned herein are incorporated by reference in their entireties for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

Definitions

[0128] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application including the definitions will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0129] To the extent that section headings are used, they should not be construed as necessarily limiting.

[0130] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof. The terms "a," "an," "the," "one or more," and "at least one," for example, can be used interchangeably herein.

[0131] As used herein, the term "about," when used to modify an amount related to the invention, refers to variation in the numerical quantity that can occur, for example, through routine testing and handling; through inadvertent error in such testing and handling; through differences in the manufacture, source, or purity of ingredients employed in the invention; and the like. Whether or not modified by the term "about", the claims include equivalents of the recited quantities. In some aspects, the term "about" means plus or minus 10% of the reported numerical value.

[0132] Throughout this application, various aspects of this invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to

have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range, such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 2, from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 3, from 2 to 4, from 2 to 5, from 2 to 6, from 3 to 4, from 3 to 5, from 3 to 6, etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0133] The terms "comprises," "comprising," "includes," "including," "having," and their conjugates are interchangeable and mean "including but not limited to." It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0134] The term "consisting of" means "including and limited to."

[0135] The term "consisting essentially of" means the specified material of a composition, or the specified steps of a method, and those additional materials or steps that do not materially affect the basic characteristics of the material or method.

[0136] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0137] The terms "culture," "to culture," and "culturing," as used herein, means to incubate cells under *in vitro* conditions that allow for cell growth or division or to maintain cells in a living state. The term "a culture" can also be used herein to refer to cells incubated under *in vitro* conditions (e.g., cells incubated a liquid growth media).

[0138] The term "probiotic" as used herein refers to one or more live microorganisms (bacteria and/or yeast) and may or may not include other ingredients, which when administered in adequate amounts may confer a health benefit on the animal or subject.

[0139] The term "direct-fed microbial product" as used in herein refers to a product that contains one or more live microorganisms (bacteria and/or yeast) and may or may not include other ingredients, that can be administered to the animal or subject in feed

mixtures, boluses, and/or oral pastes, and when administered in adequate amounts may confer a health benefit on the animal or subject.

- [0140] The term "feed additive" as used herein refers to one or more ingredients, products, or substances (e.g., cells), used alone or together, in nutrition (e.g., to improve the quality of a food (e.g., an animal feed), to improve an animal's performance and health, and/or to enhance digestibility of a food or materials within a food). A feed additive can be, for example, a probiotic.
- [0141] The terms "growth media" and "culture media" as used herein refer to a solid (e.g., agar), semi-solid (e.g., agar), or liquid (e.g., broth) composition that contains components to support the growth of cells.
- [0142] The terms "harvest" and "harvesting" as used herein refer to collecting cells from a culture, e.g., collecting cells in growth media from the culture, collecting cells by removing an amount of the growth media from the cells (e.g., by concentrating the cells in a liquid culture or separating the cells from the growth media), or halting the culturing of the cells. The terms include collecting or removing a volume of liquid comprising the cells from a liquid culture, including a volume in which the cells have been concentrated.
- [0143] The term "isolated" as used herein does not necessarily reflect the extent to which an isolate has been purified but indicates isolation or separation from a native form or native environment. An isolate can include, but is not limited to, an isolated microorganism, an isolated biomass, or an isolated culture.
- [0144] As used herein, "excipient" refers to a component, or mixture of components, that is used to give desirable characteristics to a feed additive, food, composition, or pharmaceutical composition as disclosed herein. An excipient of the present invention can be described as a "pharmaceutically acceptable" excipient when added to a pharmaceutical composition, meaning that the excipient is a compound, material, composition, salt, and/or dosage form which is, within the scope of sound medical judgment, suitable for contact with tissues of animals (i.e., human and non-human animals) without excessive toxicity, irritation, allergic response, or other problematic complications over the desired duration of contact commensurate with a reasonable benefit/risk ratio.

- [0145] As used herein, the term "yield" refers to the amount of living, or viable, cells, including the amount in a particular volume (e.g., colony-forming units per milliliter ("CFU/mL")) or in a particular weight (e.g., CFU per gram ("CFU/g")).
- [0146] As used herein, the term "viable" refers to a living organism or organisms (e.g., a microbial cell that is alive or microbial cells that are alive). "Viability" refers to the ability to live, especially under certain conditions.
- [0147] As used herein, "purify," "purified," and "purification" mean to make substantially pure or clear from unwanted components, material defilement, admixture or imperfection.
- [0148] As used herein, the terms "animal" or "subject" refer to any organism belonging to the kingdom Animalia and includes without limitation, unless otherwise noted, aquatic animals and terrestrial animals such as fish; commercial fish; ornamental fish; fish larvae; bivalves; mollusks; crustaceans; shellfish; shrimp; larval shrimp; artemia; rotifers; brine shrimp; filter feeders; amphibians; reptiles; mammals; non-human animals; domestic animals; farm animals; zoo animals; sport animals; breeding stock; racing animals; show animals; heirloom animals; rare or endangered animals; companion animals; pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, or horses; primates such as monkeys (e.g., cebus, rhesus, African green, patas, cynomolgus, and cercopithecus), apes, orangutans, baboons, gibbons, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equids such as horses, ponies, donkeys, mules, and zebras; food animals such as cows, buffalo, cattle, pigs, poultry, and sheep; ungulates such as deer and giraffes; avians (i.e., birds); poultry such as chickens, geese, ducks, quails, turkeys, pigeons, emus, ostriches, and any other bird used as a food or farm animal, including broilers, broiler breeders, and a layers; rodents such as mice, rats, hamsters and guinea pigs; and so on. In some aspects, the subject is a mammal. In some aspects, the mammal is a human subject. In some aspects, the mammal excludes a human subject. An animal feed includes, but is not limited to, an aquaculture feed, a domestic animal feed including a pet feed, a zoological animal feed, a work animal feed, a livestock feed, and combinations thereof. In some aspects, food includes animal feed and human food.
- [0149] As used herein, the term "vegetative state" refers to bacterial cells that are actively metabolizing nutrients and growing.

- [0150] As used herein, the term "spore" refers to bacterial cells that are not growing or reproducing. This state helps bacteria survive in an environment that is unfavorable for growth.
- [0151] As used here, the term "encapsulation," "encapsulate," and "encapsulated," refer to vegetative bacterial cells (e.g., *Megasphaera elsdenii* cells) that form at least one solid core, which is surrounded by at least one continuous membrane or shell. In some aspects, the core can be mixed with a carrier and coated with one or more lipids to form a core. In some aspects, the core can be coated with at least one layer or one or more lipids.
- [0152] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate aspects, can also be provided in combination in a single aspect. Conversely, various features of the invention, which are, for brevity, described in the context of a single aspect, can also be provided separately or in any suitable subcombination or as suitable in any other described aspect of the invention. Certain features described in the context of various aspects are not to be considered essential features of those aspects unless the aspect is inoperative without those elements.
- [0153] Although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention, suitable methods and materials are described below. The materials, methods and examples are illustrative only and are not intended to be limiting. Other features and advantages of the invention will be apparent from the detailed description and from the claims.

Megasphaera elsdenii

- [0154] *Megasphaera elsdenii* (*M. elsdenii*) cells from any strain or any combination of strains can be used in the disclosure described herein.
- [0155] A *M. elsdenii* strain or strains can be selected from a stock culture collection (e.g., American Type Culture Collection ("ATCC®"), National Collection of Industrial, Food and Marine Bacteria ("NCIMB"), National Collection of Type Cultures ("NCTC"), American Research Service ("ARC") culture collection (i.e., "NRRL"), National Institute of Animal Health (NIAH) culture collection, or can be a strain that has been isolated from a natural source (e.g., from the gastrointestinal tract of a ruminant).
- [0156] Examples of *M. elsdenii* strains that can be selected from a culture collection include, but are not limited to, the strains listed by deposit numbers in **Table 1**. Alternative designations of the deposit numbers are also indicated.

Table 1. Examples of *M. elsdenii* Strains and Source of Each Strain.

Deposit Number	Alternative Designations	Source of Strain
ATCC® 25940	NCIMB 8927; BE2-2083	Rumen of Sheep
ATCC® 17752	B159; NCIMB 702409; NCDO2409	N/A
ATCC® 17753	T81; NCIMB 702410; NCDO2410	N/A
NCIMB 702261	A17-2; A12-2; NCDO2261	Human Adult Feces
NCIMB 702262	S17-3; NCDO2262	Juvenile Swine Feces
NCIMB 702264	LC1	N/A
NCIMB 702331	LC1; NCDO2263; NCDO2264; NCDO2331	N/A
NCIMB 41125	CH4	Rumen of Dairy Cow
NCIMB 41787	CH3	Rumen of Dairy Cow
NCIMB 41788	CH7	Rumen of Dairy Cow
NRRL 18624		Rumen of Bovine
NIAH 1102		N/A
CBS146325 (NRRL)		
CBS146326 (NRRL)		
CBS146327 (NRRL)		
CBS146328 (NRRL)		
CBS146329 (NRRL)		
CBS146330 (NRRL)		

[0157] In some aspects, the *M. elsdenii* cells are from a strain having a deposit number selected from the group consisting of: ATCC® 25940, ATCC® 17752, ATCC® 17753, NCIMB 702261, NCIMB 702262, NCIMB 702264, NCIMB 702331, NCIMB 702409, NCIMB 702410, NCIMB 41125, NCIMB 41787, NCIMB 41788, NRRL 18624, NIAH 1102, CBS146325, CBS146326, CBS146327, CBS146328, CBS146329, CBS146330, and combinations thereof, including any of the alternative designations in Table 1 or any commercially available sources of *M. elsdenii*.

[0158] In some aspects, the *M. elsdenii* cells are from a strain isolated from a ruminant (e.g., a cow). *See*, e.g., U.S. Patent No. 7,550,139.

[0159] In some aspects, the *M. elsdenii* cells are from a strain isolated from a non-ruminant (e.g., a human).

[0160] In some aspects, the *M. elsdenii* cells are from a strain selected for lactate utilization (e.g., a strain that utilizes lactate in the presence of sugars), resistance to ionophore antibiotics, relatively high growth rate, capability to produce predominantly acetate, capability to proliferate at pH values below 5.0 and as low as 4.5, production of volatile fatty acids (VFAs), phytase activity, and combinations thereof. *See*, e.g., U.S. Patent No. 7,550,139.

- [0161] In some aspects, a strain selected for lactate utilization utilizes lactate as a preferred carbon source in the presence of a soluble carbohydrate (e.g., glucose and/or maltose). Lactate utilization can be determined, for example, based on growth in a medium containing lactate and lacking soluble carbohydrates as compared to the same medium supplemented with soluble carbohydrates.
- [0162] In some aspects, the *M. elsdenii* cells are from a strain with a high growth rate as compared to other strains. The growth rates of different strains can be determined, for example, by culturing the cells in a liquid medium and monitoring the increase in optical density over time.
- [0163] In some aspects, the *M. elsdenii* cells are from a strain with phytase activity.
- [0164] In some aspects, the *M. elsdenii* cells are from *Megasphaera elsdenii* strain NCIMB 41125. This strain of *Megasphaera elsdenii* has a high specific growth rate (0.94 generations/hour), is capable of growth in a pH range of 4.5 to 6.5 or more, uses D- and L-Lactate as its preferred substrate, but also has the ability to utilize glucose and other carbohydrates, and tolerates ionophores.
- [0165] In some aspects, the *M. elsdenii* cells are from *Megasphaera elsdenii* strain NCIMB 41787. In some aspects, the *M. elsdenii* cells are from *Megasphaera elsdenii* strain NCIMB 41788.
- [0166] In some aspects, the *M. elsdenii* cells are from *Megasphaera elsdenii* strain ATCC® 25940.
- [0167] In some aspects, the *M. elsdenii* cells are derived from a strain selected from a stock culture collection or isolated from a natural source. Cells that are "derived" from a strain can be a natural or artificial derivative such as, for example, a sub-isolate, a mutant, variant, or recombinant strain.
- [0168] In some aspects, the *M. elsdenii* are freeze-dried. See WO 2018/144653 A1, which is herein incorporated by reference in its entirety.

Preparing a Culture Comprising Anaerobic bacterial cells or *Megasphaera elsdenii* cells

- [0169] Anaerobic bacterial cells, including *M. elsdenii*, should be cultured under anaerobic conditions in order to obtain maximum yield and viability.
- [0170] In some aspects, a culture comprises *M. elsdenii* cells and a growth media.

- [0171] In some aspects, the culture comprises one or more strains of *M. elsdenii* cells. In some aspects, the culture comprises a single strain of *M. elsdenii* cells. In some aspects, the culture consists of one or more strains of *M. elsdenii* cells (i.e., the cells in the culture consist of *M. elsdenii* cells, e.g., one or more strains of *M. elsdenii* cells). In some aspects, the culture consists of a single strain of *M. elsdenii* cells.
- [0172] In some aspects, a culture comprises one or more strains of anaerobic bacterial cells and a growth media. In some aspects, the culture comprises *Bifidobacterium* cells, such as *B. breve*, *Lactobacillus* cells, such as *L. plantarum*, *Bifidobacterium* cells, such as *B. animalis* subsp. *lactis*, *Pediococcus* cells, such as *P. acidilactici*, *Lactobacillus* cells, such as *L. casei*, *Fibrobacter*, such as *F. succinogenes*, and *Butyrivibrio*, such as *B. fibrisolvens*, *Ruminococcus*, such as *Ruminococcus flavefaciens*, *Blautia* cells, such as *B. obeum*, *Clostridium* cells, such as *C. butyricum*, *Akkermansia* cells, such as *A. muciniphila* and a growth media.
- [0173] Various fermentation parameters for inoculating, growing, and harvesting anaerobic bacterial cells (e.g., *M. elsdenii* cells) can be used, including continuous fermentation (i.e., continuous culture) or batch fermentation (i.e., batch culture). See, for example, U.S. Patent No. 7,550,139.
- [0174] Growth media for anaerobic bacterial cells (e.g., *M. elsdenii* cells) can be a solid, semi-solid, or liquid. A medium can contain nutrients that provide essential elements and specific factors that enable growth. A variety of microbiological media and variations are well known in the art. Media can be added to a culture at any time, including the start of the culture, during the culture, or intermittently/continuously.
- [0175] Examples of growth media include, but are not limited to: (1) semi-defined media, which contains peptone, 3 g/L; yeast, 3 g/L; vitamin solution, 2 mL/L; mineral solution, 25 mL/L; indigo carmine (0.5%), 1 g/L; 12.5% L-cysteine, 2 g/L; 12.5% sodium sulfide, 2 g/L; and supplemented with either Na-lactate (semi-defined lactate, SDL), glucose (semi-defined glucose, SDG), or maltose (semi-defined maltose, SDM); (2) Modified Reinforced Clostridial Agar/Broth Medium (pre-reduced), which contains peptone, 10 g/L; beef extract, 10 g/L; yeast extract, 3 g/L; dextrose 5 g/L; NaCl, 5 g/L; soluble starch, 1 g/L; L-cysteine HCl, 0.5 g/L; sodium acetate, 3 g/L; and resazurin (0.025%), 4 mL/L; (3) Trypticase soy agar/broth with defibrinated sheep blood; (4) semi-defined rumen fluid free medium, which contains Na-lactate (70%), 10 g/l; Peptone, 2 g/l; KH₂PO₄ 1 g/l;

(NH₄)₂SO₄ 3 g/l; MgSO₄ 7H₂O 0.2 g/l; CaCl₂·2H₂O 0.06 g/l; Vitamins (Pyridoxolhydrochloride, 4 mg/l; Pyridoxamine, 4 mg/l; Riboflavin, 4 mg/l; Thiaminiumchloride, 4 mg/l; Nicotinamide, 4 mg/l; Ca-D-pantothenate, 4 mg/l; 4-Aminobenzoic acid, 0.2 mg/l; Biotin, 0.2 mg/l; Folic acid, 0.1 mg/l and Cyanocobalamin, 0.02 mg/l); Na₂S·9H₂O, 0.25 g/l; Cysteine, 0.25 g/l; Antifoam, 0.07 ml/l and Monensin, 10 mg/l; and which is prepared by adding the Na-lactate and mineral solution to a reservoir bottle and autoclaving for 60 minutes; dissolving the peptone in 300 ml distilled H₂O and autoclaving separately; filter sterilizing the vitamin solution and two reducing agents beforehand; following autoclaving, gassing the reservoir bottle with anaerobic gas overnight; adding the other constituents separately after cooling; and adjusting the pH to the desired value with 5N HCl; and (5) incubated rumen fluid lactate ("IRFL") medium, which contains 400 ml incubated clarified rumen fluid from lucerne-fed sheep, 371 ml distilled water, 2 g peptone, 15 g agar, 100 ml 10% (w/v) sodium-D, L-lactate solution, 100 ml 0.04% (w/v) bromocresol purple solution, and 25 ml mineral solution containing 40 g/l KH₂PO₄; 120 g/l (NH₄)₂SO₄; 8 g/l MgSO₄·7H₂O and 2.4 g/l CaCl₂·2H₂O, where lactic acid (90% w/v) is used to adjust the pH to 5.5 before autoclaving at 121° C for 25 minutes, then cooling in a 50° C water bath while being gassed with an anaerobic gas mixture, followed by adding two milliliters of each of Na₂S·9H₂O (12.5% w/v) and cysteine.HCl.H₂O (12.5% w/v).

- [0176] In some aspects, the culture comprises a growth media comprising at least two carbon sources. In some aspects, the at least two carbon sources are selected from the group consisting of: casein, starch (e.g., gelatinized starch and/or soluble starch), lactate (i.e., lactic acid), dextrose, fructose, fructan, glucose, sucrose, lactose, maltose, acetate, glycerol, mannitol, sorbitol, saccharose, xylose, molasses, fucose, glucosamine, dextran, a fat, an oil, glycerol, sodium acetate, arabinose, soy protein, soluble protein, raffinose, amylose, starch, tryptone, yeast extract and combinations thereof.
- [0177] In some aspects, the at least two carbon sources consist of about 1-99% of a first carbon source (e.g., any carbon source described herein) and about 1-99% of a second carbon source (e.g., any carbon source described herein that is different from the first carbon source), wherein 100% of the at least two carbon sources consist of the first carbon source and the second carbon source. In some aspects, the at least two carbon sources consist of about 50-60% of the first carbon source and about 40-50% of the

second carbon source, about 50-70% of the first carbon source and about 30-50% of the second carbon source, about 50-80% of the first carbon source and about 20-50% of the second carbon source, or about 50-90% of the first carbon source and about 10-50% of the second carbon source. In some aspects, the at least two carbon sources consist of about 65-75% of the first carbon source and about 25-35% of the second carbon source. In some aspects, the first carbon source is lactate.

[0178] In some aspects, the anaerobic bacterial cells (e.g., *M. elsdenii* cells) are grown at about 39 °C to about 40 °C, at about 35 °C, at about 36 °C, at about 37 °C, at about 38 °C, at about 39 °C, or at about 40 °C.

[0179] In some aspects, the anaerobic bacterial cells (e.g., *M. elsdenii* cells) are grown at about 35 °C to about 40 °C.

[0180] To culture anaerobic bacterial cells (e.g., *M. elsdenii* cells), fermenters of different sizes and designs that maintain anaerobic conditions can be used. A fermenter can be capable, for example, of fermenting culture volumes sufficient for commercial production of the anaerobic cells (e.g., *M. elsdenii* cells). In some aspects, the culture volume is about 2 liters, about 10 liters, about 50 liters, about 100 liters, about 150 liters, about 200 liters, about 250 liters, about 300 liters, about 350 liters, about 400 liters, about 450 liters, about 500 liters, about 600 liters, about 800 liters, about 1,000 liters, about 1,200 liters, about 1,500 liters, about 1,800 liters, about 2,000 liters, about 2,200 liters, about 2,500 liters, about 2,750 liters, about 3,000 liters, about 4,000 liters, about 5,000 liters, about 6,000 liters, about 7,000 liters, about 8,000 liters, about 9,000 liters, about 10,000 liters, at least about 20,000 liters, at least about 50,000 liters, or at least about 75,000 liters. In some aspects, the fermentation volume is about 2 liters to about 75,000 liters, about 250 liters to about 750 liters, about 300 liters to about 800 liters, about 350 liters to about 850 liters, about 400 liters to about 900 liters, about 450 liters to about 950 liters, about 500 liters to about 1,000 liters, about 750 liters to about 1,250 liters, about 1,000 liters to about 2,000 liters, about 2,000 liters to about 4,000 liters, about 4,000 liters to about 8,000 liters, about 5,000 liters to about 10,000 liters, about 50 liters to about 75,000 liters, about 50 liters to about 50,000 liters, about 50 liters to about 25,000 liters, about 50 liters to about 20,000 liters, about 50 liters to about 15,000 liters, about 50 liters to about 10,000 liters, about 100 liters to about 10,000 liters, about 100 liters to about 5,000 liters, about 100 liters to about 4,000 liters, about 100 liters to about 3,000 liters, about 100

liters to about 2,900 liters, about 100 liters to about 2,850 liters, about 100 liters to about 2,800 liters, about 100 liters to about 2,750 liters, about 2 liters, about 10 liters, about 50 liters, about 100 liters, about 200 liters, about 500 liters, about 1,000 liters, about 1,500 liters, about 10,000 liters, about 20,000 liters, about 50,000 liters, or about 75,000 liters.

[0181] In some aspects, the culture comprises a liquid, and the method comprises harvesting the anaerobic bacterial cells (e.g., *M. elsdenii* cells) by removing a percentage of the liquid. In some aspects, harvesting the cells comprises removing about 5% to about 100%, about 10% to about 100%, about 15% to about 100%, about 20% to about 100%, about 25% to about 100%, about 30% to about 100%, about 35% to about 100%, about 40% to about 100%, about 45% to about 100%, about 50% to about 100% of the liquid, about 55% to about 100%, about 60% to about 100%, about 65% to about 100%, about 70% to about 100%, about 75% to about 100%, about 80% to about 100%, about 85% to about 100%, about 90% to about 100%, or about 95% to about 100% of the liquid. In some aspects, harvesting the cells comprises removing at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 100% of the liquid.

[0182] In some aspects, the method comprises harvesting the anaerobic bacterial cells (e.g., *M. elsdenii* cells) by concentrating the cells. In some aspects, harvesting the cells comprises concentrating the cells by at least one technique selected from the group consisting of: centrifugation, filtration, dialysis, reverse osmosis, and combinations thereof. In some aspects, the filtration comprises clay filtration. In some aspects, the filtration comprises tangential flow filtration, also known as cross-flow filtration.

[0183] In some aspects, the pH of the culture comprising the anaerobic bacterial cells (e.g., *M. elsdenii* cells) at the time of harvesting is between about 4.5 to about 7.0, between about 4.5 to about 6.5, between about 4.5 to about 6.0, between about 4.5 to about 5.5, between about 4.5 to about 5.0, between about 4.6 to about 6.9, between about 4.7 to about 6.8, between about 4.8 to about 6.7, between about 4.9 to about 6.6, between

about 5.0 to about 7.0, between about 5.0 to about 6.5, between about 5.0 to about 6.0, between about 5.0 to about 5.5, between about 5.1 to about 6.9, between about 5.2 to about 6.8, between about 5.3 to about 6.7, between about 5.4 to about 6.6, between about 5.5 to about 7.0, between about 5.5 to about 6.5, between about 5.1 to about 6.4, between about 5.2 to about 6.3, between about 5.3 to about 6.2, between about 5.4 to about 6.1, between about 5.5 to about 6.0, between about 5.0 to about 6.1, between about 5.0 to about 6.2, between about 5.0 to about 6.3, between about 5.0 to about 6.4, between about 5.1 to about 6.5, between about 5.2 to about 6.5, between about 5.3 to about 6.5, or between about 5.4 to about 6.5.

[0184] In some aspects, the method comprises inoculating growth media in a fermenter with an inoculum comprising anaerobic bacterial cells (e.g., *M. elsdenii* cells) to prepare a culture, and incubating the culture at a temperature of about 39° C until the pH of the culture is about 6.0. In some aspects, the inoculum comprising anaerobic bacterial cells (e.g., *M. elsdenii* cells) is a flask culture of anaerobic cells (e.g., *M. elsdenii* cells) or a portion thereof. In some aspects, the method comprises inoculating growth media in a fermenter an inoculum to media ratio of 1/50 to 1/4,000. In some aspects, the inoculum to media ratio is 1/100.

Encapsulated anaerobic bacteria and *Megasphaera elsdenii* cells

[0185] To help animals (e.g., cattle) avoid conditions associated with changing diets from a roughage diet to a high starch diet, *Megasphaera elsdenii* cells can be administered to animals. Because anaerobic bacterial cells and *Megasphaera elsdenii* cells cannot survive in the presence of oxygen, it is difficult to maintain viability until the cells can be administered. Thus, the present disclosure provides encapsulation formulations that maintain viability of the cells for storage at various temperatures (e.g., about -20° C, about 4° C, about 25° C, or about 52° C), at various pH levels (e.g., about 4.0, about 4.3, about 4.5, about 4.7, about 5, about 5.2, about 5.4, about 5.6, about 5.8, or about 6.0), and various moisture levels (e.g., about 5%, about 9%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, or about 45%).

[0186] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single

layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells are viable in the composition.

[0187] In some aspects, the compositions disclosed herein are formulated for delivery to the rumen and release in the rumen of a ruminant animal.

[0188] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a feed or feed additive.

[0189] In some aspects, the *Megasphaera elsdenii* cells in the composition are viable for up to 48 hours at 25° C and pH 7.0 after being added to the feed or feed additive.

[0190] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, administration of the composition to a ruminant results in viable bacterial cells being released in the rumen.

[0191] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a temperature of at least 40° C to 60° C at pH 7.0 for 4-18 hours.

[0192] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is

coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a pH between 3-7 at 25° C for up to 48 hours.

[0193] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a pH between 3-7 and a temperature between 25° C to 60° C for up to 48 hours.

[0194] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being processed through a micromachine system.

[0195] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for up to 14 hours.

[0196] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single

layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for up to 48 hours.

- [0197] In some aspects, at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for about 14 hours to about 48 hours.
- [0198] In some aspects, the at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, about 24 hours, about 26 hours, about 28 hours, about 30 hours, about 32 hours, about 34 hours, about 36 hours, about 38 hours, about 40 hours, about 42 hours, about 44 hours, about 46 hours, or about 48 hours.
- [0199] In some aspects, the at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for at least about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, about 24 hours, about 26 hours, about 28 hours, about 30 hours, about 32 hours, about 34 hours, about 36 hours, about 38 hours, about 40 hours, about 42 hours, about 44 hours, about 46 hours, or about 48 hours.
- [0200] In some aspects, the at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for about 1 hour to about 48 hours, about 2 hours to about 48 hours, about 4 hours to about 48 hours, about 6 hours to about 48 hours, about 8 hours to about 48 hours, about 10 hours to about 48 hours, about 12 hours to about 48 hours, about 14 hours to about 48 hours, about 16 hours to about 48 hours, about 18 hours to about 48 hours, about 20 hours to about 48 hours, about 22 hours to about 48 hours, about 24 hours to about 48 hours, about 26 hours to about 48 hours, about 28 hours to about 48 hours, about 30 hours to about 48 hours, about 32 hours to about 48 hours, about 34 hours to about 48 hours, about 36 hours to about 48 hours, about 38 hours to about 48 hours, about 40 hours to about 48 hours, about

42 hours to about 48 hours, about 44 hours to about 48 hours, about 46 hours to about 48 hours, or about 48 hours.

[0201] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are viable in the composition.

[0202] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a feed or feed additive.

[0203] In some aspects, the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable for up to 48 hours at 25° C and pH 7.0 after being added to the feed or feed additive.

[0204] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, the administration of the composition to a ruminant results in viable bacterial cells being released in the rumen.

[0205] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one

or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a temperature of 40° C to 60° C at pH 7.0 for 4-18 hours.

[0206] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a pH between 3-7 at 25° C for up to 48 hours.

[0207] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a pH between 3-7 and a temperature between 25° C to 60° C for up to 48 hours.

[0208] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a micromachine.

- [0209] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine for up to 14 hours. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine prior to addition to feedtruck for up to 14 hours.
- [0210] In some aspects, the present disclosure provides a composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids. In some aspects, the core is coated by the layer of one or more lipids. In some aspects, the core is coated by a single layer of one or more lipids. In some aspects, the core is coated by two or more layers of one or more lipids. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine for up to 48 hours. In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine prior to addition to feedtruck for up to about 48 hours.
- [0211] In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine prior to addition to feedtruck for about 14 hours to about 48 hours.
- [0212] In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine for up to about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, about 24 hours, about 26 hours, about 28 hours, about 30 hours, about 32 hours, about 34 hours, about 36 hours, about 38 hours, about 40 hours, about 42 hours, about 44 hours, about 46 hours, or about 48 hours.

- [0213] In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine for at least about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, about 24 hours, about 26 hours, about 28 hours, about 30 hours, about 32 hours, about 34 hours, about 36 hours, about 38 hours, about 40 hours, about 42 hours, about 44 hours, about 46 hours, or about 48 hours.
- [0214] In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine prior to addition to feedtruck for about 1 hour, about 2 hours, about 4 hours, about 6 hours, about 8 hours, about 10 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, about 24 hours, about 26 hours, about 28 hours, about 30 hours, about 32 hours, about 34 hours, about 36 hours, about 38 hours, about 40 hours, about 42 hours, about 44 hours, about 46 hours, or about 48 hours.
- [0215] In some aspects, at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine prior to addition to feedtruck for about 1 hour to about 48 hours, about 2 hours to about 48 hours, about 4 hours to about 48 hours, about 6 hours to about 48 hours, about 8 hours to about 48 hours, about 10 hours to about 48 hours, about 12 hours to about 48 hours, about 14 hours to about 48 hours, about 16 hours to about 48 hours, about 18 hours to about 48 hours, about 20 hours to about 48 hours, about 22 hours to about 48 hours, about 24 hours to about 48 hours, about 26 hours to about 48 hours, about 28 hours to about 48 hours, about 30 hours to about 48 hours, about 32 hours to about 48 hours, about 34 hours to about 48 hours, about 36 hours to about 48 hours, about 38 hours to about 48 hours, about 40 hours to about 48 hours, about 42 hours to about 48 hours, about 44 hours to about 48 hours, about 46 hours to about 48 hours, or about 48 hours.
- [0216] In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1600 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1500 μm . In some aspects, the particle size of the

Megasphaera elsdenii cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1400 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1300 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1200 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1100 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1000 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 900 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 800 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 700 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 600 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 500 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 400 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 300 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 200 μm . In some aspects, the particle size of the *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 100 μm .

[0217] In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1600 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1500 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1400 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1300 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1200 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1100 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 1000 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 900 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 800 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 700 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 600 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 500 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 400 μm . In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in

a vegetative state in the composition are less than about 300 µm. In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 200 µm. In some aspects, the particle size of the freeze-dried *Megasphaera elsdenii* cells, anaerobic bacterial cells, or anaerobic bacterial cells in a vegetative state in the composition are less than about 100 µm.

[0218] In some aspects, at least 15%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 99% of the *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are viable in the composition.

[0219] In some aspects, about 15%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are viable in the composition. In some aspects, between about 15% and about 99%, about 15% and about 90%, about 15% and about 80%, about 15% and about 70%, about 15% and about 60%, about 15% and about 50%, about 15% and about 40%, about 15% and about 30%, about 15% and about 20%, about 20% and about 90%, about 30% and about 90%, about 40% and about 90%, about 50% and about 90%, about 60% and about 90%, about 70% and about 90%, about 80% and about 90%, or about 30% and about 60% of the *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are viable in the composition.

[0220] In some aspects, the *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are dried.

[0221] In some aspects, the *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are dried by spray drying, electrospray drying, vacuum drying, jet drying, freeze-drying, or combinations thereof.

[0222] In some aspects, the composition comprises from about 0.1% to about 15% (w/w) *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state.

[0223] In some aspects, the composition comprises from about 0.1% to about 15% (w/w), about 1% to about 15% (w/w), about 2% to about 15% (w/w), about 3% to about 15% (w/w), about 4% to about 15% (w/w), about 5% to about 15% (w/w), about 6% to about

15% (w/w), about 7% to about 15% (w/w), about 8% to about 15% (w/w), about 9% to about 15% (w/w), about 10% to about 15% (w/w), about 11% to about 15% (w/w), about 12% to about 15% (w/w), about 13% to about 15% (w/w), about 14% to about 15% (w/w), about 0.1% to about 10% (w/w), about 1% to about 10% (w/w), about 5% to about 10% (w/w), about 0.1% to about 5% (w/w), about 1% to about 5% (w/w), or about 0.1% to about 1% (w/w) *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state.

[0224] In some aspects, the composition comprises at least about 0.1%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about 10%, about 11%, about 12%, about 13%, about 14%, or about 15% (w/w) *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state.

[0225] In some aspects, the composition comprises *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state from about 1×10^4 to about 1×10^{10} CFU/g.

[0226] In some aspects, the composition comprises *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state from about 1×10^4 to about 1×10^{10} CFU/g, about 1×10^5 to about 1×10^{10} CFU/g, about 1×10^6 to about 1×10^{10} CFU/g, about 1×10^7 to about 1×10^{10} CFU/g, about 1×10^8 to about 1×10^{10} CFU/g, about 1×10^9 to about 1×10^{10} CFU/g, 1×10^4 to about 1×10^9 CFU/g, 1×10^4 to about 1×10^8 CFU/g, 1×10^4 to about 1×10^7 CFU/g, 1×10^4 to about 1×10^6 CFU/g, 1×10^4 to about 1×10^5 CFU/g, or 1×10^6 to about 1×10^8 CFU/g.

[0227] In some aspects, the composition comprises about 1×10^4 CFU/g, about 1×10^5 CFU/g, about 1×10^6 CFU/g, about 1×10^7 CFU/g, about 1×10^8 CFU/g, about 1×10^9 CFU/g, or about 1×10^{10} CFU/g *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state.

[0228] In some aspects, the anaerobic bacteria or anaerobic bacterial cells in a vegetative state are selected from the group consisting of *Bifidobacterium breve*, *Lactobacillus plantarum*, *Bifidobacterium animalis subsp. lactis*, *Pediococcus acidilactici*, *Lactobacillus casei*, *Megasphaera elsdenii*, *Fibrobacter succinogenes*, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, *Blautia obeum*, *Clostridium butyricum*, *Akkermansia muciniphila* and combinations thereof.

- [0229] In some aspects, the composition is a granule, capsule, minicapsule, microcapsule, tablet, minitab, or microtablet.
- [0230] In some aspects, the composition has a moisture content of about 5% (w/w) or less.
- [0231] In some aspects, the composition has a moisture content of about 5% (w/w), about 4%, about 3%, about 2%, about 1%, about 0.5%, or about 0.1%.
- [0232] In some aspects, the one or more lipids in the core is selected from the group consisting of animal oils or fats, vegetable oils or fats, triglycerides, free fatty acids, animal waxes, beeswax, lanolin, shell wax, Chinese insect wax, vegetable waxes, carnauba wax, candelilla wax, bayberry wax, sugarcane wax, mineral waxes, synthetic waxes, natural and synthetic resins, and mixtures thereof.
- [0233] In some aspects, the one or more lipids in the core is an animal fat or oil and/or a plant fat or oil.
- [0234] In some aspects, the plant fat or oil is selected from the group consisting of canola oil, cottonseed oil, hydrogenated cottonseed oil, peanut oil, corn oil, olive oil, soybean oil, hydrogenated soybean oil, sunflower oil, safflower oil, coconut oil, palm oil, hydrogenated palm oil, linseed oil, tung oil, castor oil and rapeseed oil.
- [0235] In some aspects, the plant fat or oil is hydrogenated palm oil.
- [0236] In some aspects, the free fatty acid is myristic acid, lauric acid, or stearic acid, or combinations thereof.
- [0237] In some aspects, *Megasphaera elsdenii*, anaerobic bacteria, or anaerobic bacterial cells in a vegetative state can be encapsulated using one or more lipids disclosed herein with the spinning disk encapsulation method to form a core. Using the spinning disk encapsulation method, a spray of droplets with a narrow size range can produce a core of cells that has been coated with one or more lipids. Droplet formation depends on fluid flow velocity exited from the nozzle. Droplet formation may occur directly from the coincidence of liquid flow with the edge of the rotating disk or indirectly through the dispersion of ligament or sheet produced from fluid flow over the rotating disk. Many parameters should be considered in the spinning disk encapsulation process, including liquid flow rate, viscosity, and mass transfer phenomena from microcapsule.
- [0238] In some aspects, the Spinning disk apparatus comprises a spinning disk/cup rotating on its central axis to form droplets and projecting them radially outward, and

collection basin(s) surrounding the spinning cup to collect the core beads projected from the cup.

- [0239] In some aspects, on the day of processing, maltodextrin (5 parts) is mixed with hydrogenated palm oil (HPO) (coating lipid; 11.2 parts) at 70°C-80°C, until a homogeneous mixture is obtained. Dried powder (1 part) (e.g., freeze-dried powder or electrospray dried powder) (1 part) is then added to the mixture (maltodextrin and HPO) at 60°C-65°C temperature and flowed through the spinning disk/cup causing droplets to be projected radially outwards. The droplets instantly solidify while cooling to below 55°C to form the core (FD *M. elsdonii*, maltodextrin, and HPO) and the core beads are collected into the collection basin.
- [0240] In some aspects, the one or more lipids in the core has a melting point of about 40° C to about 85° C.
- [0241] In some aspects, the one or more lipids in the core has a melting point of about 55° C to about 75° C.
- [0242] In some aspects, the one or more lipids in the core has a melting point of about 40° C to about 85° C, about 45° C to about 85° C, about 50° C to about 85° C, about 55° C to about 85° C, about 60° C to about 85° C, about 65° C to about 85° C, about 70° C to about 85° C, about 75° C to about 85° C, about 80° C to about 85° C, about 55° C to about 75° C, about 60° C to about 75° C, about 65° C to about 75° C, about 70° C to about 75° C, about 40° C to about 80° C, about 40° C to about 75° C, about 40° C to about 70° C, about 40° C to about 65° C, about 40° C to about 60° C, about 40° C to about 55° C, about 40° C to about 50° C, about 40° C to about 45° C, about 55° C to about 70° C, about 55° C to about 65° C, or about 55° C to about 60° C.
- [0243] In some aspects, the one or more lipids in the core has a melting point of about 40° C, about 45° C, about 50° C, about 55° C, about 60° C, about 65° C, about 70° C, about 75° C, about 80° C, or about 85° C.
- [0244] In some aspects, the one or more lipids coating the core is selected from the group consisting of animal oils or fats, vegetable oils or fats, triglycerides, free fatty acids, animal waxes, beeswax, lanolin, shell wax, Chinese insect wax, vegetable waxes, carnauba wax, candelilla wax, bayberry wax, sugarcane wax, mineral waxes, synthetic waxes, natural and synthetic resins, and mixtures thereof.

- [0245] In some aspects, the one or more lipids coating the core is an animal fat or oil and/or a plant fat or oil.
- [0246] In some aspects, the plant fat or oil is selected from the group consisting of cottonseed oil, hydrogenated cottonseed oil, peanut oil, corn oil, olive oil, soybean oil, hydrogenated soybean oil, sunflower oil, safflower oil, coconut oil, palm oil, hydrogenated palm oil, linseed oil, tung oil, castor oil and rapeseed oil.
- [0247] In some aspects, the plant fat or oil is hydrogenated palm oil.
- [0248] In some aspects, the free fatty acid is myristic acid, lauric acid, or stearic acid.
- [0249] In some aspects, *Megasphaera elsdenii*, anaerobic bacteria, or anaerobic bacterial cells in a vegetative state in the core can be encapsulated using one or more lipids disclosed herein with a Wurster fluid bed agglomerating coater. Wurster technology is characterized by the location of a spray nozzle at the bottom of the fluidized bed. The particles are moved with a fluidizing air stream that is designed to induce a cyclic upward flow of particles, past the spray nozzle. The nozzle sprays atomized droplets of coating solution or suspension concurrently with the particle flow, depositing droplets on the surfaces of the particle as they pass upward into an expansion chamber. This expansion chamber reduces air velocity to allow particles to circulate back to the coating chamber. It also allows particles to temporarily separate from one another, minimizing the potential for particle agglomeration and accretion. Organic or aqueous coating solutions evaporate as the particles move into and through the expansion chamber, leaving non-volatile coating formulation ingredients on the particle surface as part of the developing film coat. Process parameters are designed for optimal solution evaporation and film coat characteristics. This batch process is continued until each particle is uniformly coated to the desired coat percentage or film thickness. Moreover, the Wurster fluid bed process can be used to apply a hot melt coating, such as lipids. Lipids are heated to a molten state and sprayed in the same manner as a solution suspension. Process parameters are adjusted to congeal molten lipid droplets on the surfaces of the circulating particles.
- [0250] In some aspects, the Wurster fluid bed agglomeration coater has the particularity of having a spray nozzle located at the bottom of a cylindrical Wurster tube within a chamber. The core particles (beads) are driven from the fluidized bed to the cylindrical tube thanks to differential air streams and move through the chamber in a cyclic motion,

periodically crossing the spraying zone where they collide with small droplets of the coating solution (Hydrogenated Cottonseed Oil; "HCO") to create a core-shell structure.

- [0251] In some aspects, the collected core beads are then spray coated in a Wurster fluid bed agglomeration coater with hydrogenated cottonseed oil (HCO) to obtain the final *M. elsdenii* product. The core beads are driven from the fluidized bed to the cylindrical Wurster tube thanks to differential air streams and move through the chamber in a cyclic motion, periodically crossing the spraying zone where they collide with small droplets of the coating solution (HCO) to create a core-shell structure (17.24 parts core and 5.75 parts coating).
- [0252] In some aspects, the one or more lipids coating the core has a melting point of about 55° C to about 80° C.
- [0253] In some aspects, the one or more lipids coating the core has a melting point of about 55° C to about 75° C.
- [0254] In some aspects, the one or more lipids coating the core has a melting point of about 55° C to about 80° C, about 60° C to about 80° C, 65° C to about 80° C, 70° C to about 80° C, 75° C to about 80° C, 55° C to about 75° C, 55° C to about 70° C, 55° C to about 65° C, 55° C to about 60° C, about 55° C to about 75° C, about 60° C to about 75° C, about 65° C to about 75° C, about 70° C to about 75° C, about 55° C to about 70° C, about 55° C to about 65° C, or about 55° C to about 60° C.
- [0255] In some aspects, the one or more lipids coating the core has a melting point of about 55° C, about 60° C, about 65° C, about 70° C, about 75° C, or about 80° C.
- [0256] In some aspects, the at least one carrier comprises maltodextrin, sucrose, starch, cellulose, clay, biochar, lignin-derivatives, sugar alcohols, or combinations thereof.
- [0257] In some aspects, the composition comprises a total of about 10% to about 99% (w/w) lipids.
- [0258] In some aspects, the composition comprises a total of about 70% to about 80% (w/w) lipids. In some aspects, the composition comprises a total of about 60% to about 80% (w/w) lipids.
- [0259] In some aspects, the composition comprises a total of about 10% to about 99% (w/w) lipids, about 20% to about 99% (w/w) lipids, about 30% to about 99% (w/w) lipids, about 40% to about 99% (w/w) lipids, about 50% to about 99% (w/w) lipids, about 60% to about 99% (w/w) lipids, about 70% to about 99% (w/w) lipids, about 80% to about

99% (w/w) lipids, about 90% to about 99% (w/w) lipids, about 10% to about 90% (w/w) lipids, about 10% to about 80% (w/w) lipids, about 10% to about 70% (w/w) lipids, about 10% to about 60% (w/w) lipids, about 10% to about 50% (w/w) lipids, about 10% to about 40% (w/w) lipids, about 10% to about 30% (w/w) lipids, about 10% to about 20% (w/w) lipids, about 40% to about 60% (w/w) lipids, about 30% to about 70% (w/w) lipids, or 20% to about 80% (w/w) lipids.

[0260] In some aspects, the composition comprises a total of at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% (w/w) lipids. In some aspects, the composition comprises a total of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99% (w/w) lipids.

[0261] In some aspects, the composition comprises from about 10% to about 30% (w/w) of at least one carrier.

[0262] In some aspects, the composition comprises from about 15% to about 25% (w/w) of at least one carrier.

[0263] In some aspects, the composition comprises from about 10% to about 30% (w/w), about 15% to about 30% (w/w), about 20% to about 30% (w/w), about 25% to about 30% (w/w), about 10% to about 25% (w/w), about 10% to about 20% (w/w), about 10% to about 15% (w/w), about 15% to about 25% (w/w), about 20% to about 25% (w/w), or about 15% to about 20% (w/w) of at least one carrier.

[0264] In some aspects, the composition is from about 0.1 mm to about 3 mm diameter in size.

[0265] In some aspects, the composition is from about 0.2 mm to about 0.6 mm diameter in size.

[0266] In some aspects, the composition is from about 0.2 mm to about 0.4 mm diameter in size.

[0267] In some aspects, the composition is from about 0.1 mm to about 3 mm, about 0.2 mm to about 3 mm, about 0.5 mm to about 3 mm, about 1 mm to about 3 mm, about 1.5 mm to about 3 mm, about 2 mm to about 3 mm, about 2.5 mm to about 3 mm, about 0.1 mm to about 2.5 mm, about 0.1 mm to about 2 mm, about 0.1 mm to about 1.5 mm, about 0.1 mm to about 1 mm, about 0.1 mm to about 0.9 mm, about 0.1 mm to about 0.8 mm, about 0.1 mm to about 0.7 mm, about 0.1 mm to about 0.6 mm, about 0.1 mm to about 0.5 mm, about 0.1 mm to about 0.4 mm, about 0.1 mm to about 0.3 mm, about 0.1 mm to

about 0.2 mm, about 0.2 mm to about 3 mm, about 0.2 mm to about 2.5 mm, about 0.2 mm to about 2 mm, about 0.2 mm to about 1.5 mm, about 0.2 mm to about 1 mm, about 0.2 mm to about 0.9 mm, about 0.2 mm to about 0.8 mm, about 0.2 mm to about 0.7 mm, about 0.2 mm to about 0.6 mm, about 0.2 mm to about 0.5 mm, about 0.2 mm to about 0.4 mm, or about 0.2 mm to about 0.3 mm diameter in size.

[0268] In some aspects, the composition is about 0.1 mm, about 0.2 mm, about 0.3 mm, about 0.4 mm, about 0.5 mm, about 0.6 mm, about 0.7 mm, about 0.8 mm, about 0.9 mm, about 1 mm, about 1.5 mm, about 2 mm, about 2.5 mm, or about 3 mm diameter in size.

[0269] In some aspects, the core comprises from about 1% to about 99% of the composition.

[0270] In some aspects, the core comprises from about 10% to about 90% of the composition.

[0271] In some aspects, the core comprises from about 25% to about 80% of the composition.

[0272] In some aspects, the core comprises at least about 10% to about 90%, about 20% to about 90%, about 30% to about 90%, about 40% to about 90%, about 50% to about 90%, about 60% to about 90%, about 70% to about 90%, about 80% to about 90%, about 10% to about 80%, about 10% to about 70%, about 10% to about 60%, about 10% to about 50%, about 10% to about 40%, about 10% to about 30%, about 10% to about 20%, about 25% to about 80%, about 25% to about 70%, about 25% to about 60%, about 25% to about 50%, about 25% to about 40%, about 25% to about 30%, about 30% to about 80%, about 40% to about 80%, about 50% to about 80%, about 60% to about 80%, or about 70% to about 80% of the composition. In some aspects, the core comprises at least 10% to 90%, 20% to 90%, 30% to 90%, 40% to 90%, 50% to 90%, 60% to 90%, 70% to 90%, 80% to 90%, 10% to 80%, 10% to 70%, 10% to 60%, 10% to 50%, 10% to 40%, 10% to 30%, 10% to 20%, 25% to 80%, 25% to 70%, 25% to 60%, 25% to 50%, 25% to 40%, 25% to 30%, 30% to 80%, 40% to 80%, 50% to 80%, 60% to 80%, or 70% to 80% of the composition.

[0273] In some aspects, the core comprises at least about 10%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% of the composition. In some aspects, the core comprises at least 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the composition.

- [0274] In some aspects, the one or more lipids coating the core comprises from about 1% to about 99% of the composition.
- [0275] In some aspects, the one or more lipids coating the core comprises from about 5% to about 75% of the composition.
- [0276] In some aspects, the one or more lipids coating the core comprises from about 1% to about 99%, about 5% to about 99%, about 10% to about 99%, about 20% to about 99%, about 30% to about 99%, about 40% to about 99%, about 50% to about 99%, about 60% to about 99%, about 70% to about 99%, about 80% to about 99%, about 90% to about 99%, about 1% to about 90%, about 1% to about 80%, about 1% to about 70%, about 1% to about 60%, about 1% to about 50%, about 1% to about 40%, about 1% to about 30%, about 1% to about 20%, about 1% to about 10%, about 1% to about 5%, of the composition, about 5% to about 75%, about 5% to about 70%, about 5% to about 65%, about 5% to about 60%, about 5% to about 55%, about 5% to about 50%, about 5% to about 45%, about 5% to about 40%, about 5% to about 35%, about 5% to about 30%, about 5% to about 25%, about 5% to about 20%, about 5% to about 15%, about 5% to about 10%, about 5% to about 70%, about 5% to about 60%, about 5% to about 50%, about 5% to about 40%, about 5% to about 30%, about 5% to about 20%, or about 5% to about 10% of the composition.
- [0277] In some aspects, the one or more lipids coating the core comprises at least about 1%, about 5%, about 10%, about 20%, about 25%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or about 99% of the composition. In some aspects, the one or more lipids coating the core comprises at least 1%, 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99% of the composition.
- [0278] In some aspects, the composition has a density from about 0.6 g/mL to about 1.2 g/mL.
- [0279] In some aspects, the composition has a density of about 0.6 g/mL, about 0.7 g/mL, about 0.8 g/mL, about 0.9 g/mL, about 1.0 g/mL, about 1.1 g/mL, or about 1.2 g/mL.
- [0280] In some aspects, the composition has a porosity from about 10% to about 60%.
- [0281] In some aspects, the composition has a porosity from about 10% to about 60%, about 20% to about 60%, about 30% to about 60%, about 40% to about 60%, about 50% to about 60%, about 10% to about 50%, about 10% to about 40%, about 10% to about 30%, about 10% to about 20%, about 30% to about 40%, or about 20% to about 50%.

[0282] In some aspects, the composition has a porosity of at least about 10%, about 20%, about 30%, about 40%, about 50%, or about 60%. In some aspects, the composition has a porosity of at least 10%, 20%, 30%, 40%, 50%, or 60%.

[0283] In some aspects, the composition further comprises one or more of antibiotics, antimicrobials, anti-coccidials, antiparasitics, sulfonamides, hormones, anti-bloat compounds, adrenergic receptor modulators, a phage, a prebiotic, probiotics, enzymes, essential oils, and/or a carbohydrate immune stimulant.

Feed Additives, Feeds, Premixes, and Kits

[0284] In some aspects, provided herein is a feed additive composition comprising any of the compositions disclosed herein.

[0285] In some aspects, the feed additive composition is a powder, granulate, particulate, pellet, cake, liquid, solid, suspension, emulsion, gel, or combinations thereof.

[0286] In some aspects, provided herein is a feed comprising any of the compositions disclosed herein or any of the feed additive compositions disclosed herein.

[0287] In some aspects, the feed further comprises an animal protein, a vegetable protein, corn, soybean meal, corn dried distillers grains with solubles (cDDGS), wheat, wheat proteins, gluten, wheat by products, wheat bran, wheat dried distillers grains with solubles (wDDGS), corn by products including corn gluten meal, barley, oat, rye, triticale, full fat soy, animal by-product meals, an alcohol-soluble protein, a zein, a maize zein, a kafirin, rice, paddy rice, extruded paddy rice, a protein from oil seeds, or a combination thereof.

[0288] In some aspects, the animal protein or vegetable protein is selected from the group consisting of one or more of a gliadin or an immunogenic fragment of a gliadin, a beta-casein, a beta-lactoglobulin, glycinin, beta-conglycinin, cruciferin, napin, hordeins, keratins, feather or hair meals, collagen, whey protein, fish protein, fish meals, meat protein, egg protein, soy protein and grain protein.

[0289] In some aspects, the protein from oil seeds is selected from the group consisting of soybean seed proteins, sun flower seed proteins, rapeseed proteins, canola seed proteins and combinations thereof.

[0290] In some aspects, provided herein is a premix comprising a) any of the compositions disclosed herein, or any of the feed additive compositions disclosed herein; and b) at least one mineral and/or at least one vitamin.

- [0291] In some aspects, provided herein is a kit comprising a) i) any of the compositions provided herein; ii) any of the feed additive compositions provided herein; iii) any of the feeds provided herein; and/or iv) any of the premixes provided herein; and b) instructions for formulating and/or administering to a subject.
- [0292] In some aspects, a feed additive comprises encapsulated anaerobic cells and/or *M. elsdenii* cells as disclosed herein. In some aspects, the feed additive comprises encapsulated freeze-dried anaerobic cells and/or *M. elsdenii* cells produced by a method disclosed herein.
- [0293] In some aspects, the feed additive is a solid (i.e., "a solid feed additive") or a liquid (i.e., "a liquid feed additive"). In some aspects, the feed additive is a semi-solid or a gel (i.e., "a semi-solid or a gel feed additive"). A gel feed additive can contain an oxygen scavenger (e.g., ascorbic acid). In some aspects, the feed additive is sprayed onto the coat of the animal (i.e., "a spray on additive").
- [0294] In some aspects, a solid feed additive is a powder (e.g., a flowable powder), granule (i.e., a granulate), particle (i.e., particulate), pellet, cake, water soluble concentrate, paste, bolus, tablet, dust, a component thereof, or combinations thereof.
- [0295] In some aspects, a liquid feed additive is a solution (e.g., an aqueous, organic, or aqueous-organic solution), suspension, emulsion, drench, spray, injectable, drink (e.g., a milk replacer), a component thereof, or combinations thereof.
- [0296] In some aspects, a gel feed additive is an organogel. In some aspects, the gel feed additive is an oral gel (i.e., a gel for oral administration).
- [0297] In some aspects, the feed additive is for use as a top dress (i.e., for adding to the surface of a food or mixing with a food (e.g., an animal feed)). In some aspects, the feed additive is for administration as a liquid.
- [0298] In some aspects, encapsulated anaerobic cells (e.g., *Bifidobacterium* cells, such as *B. breve*, *Lactobacillus* cells, such as *L. plantarum*, *Bifidobacterium* cells, such as *B. animalis* subsp. *lactis*, *Pediococcus* cells, such as *P. acidilactici*, *Lactobacillus* cells, such as *L. casei*, *Fibrobacter*, such as *F. succinogenes*, and *Butyrivibrio*, such as *B. fibrisolvens*, *Ruminococcus* cells, such as *R. flavefaciens*, *Blautia* cells, such as *B. obeum*, *Clostridium* cells, such as *C. butyricum*, *Akkermansia* cells, such as *A. muciniphila* and/or *Megasphaera elsdenii* cells) as disclosed herein can be used as a liquid feed additive by rehydrating, dissolving, solubilizing, and/or suspending the cells in a liquid.

- [0299] In some aspects, the feed additive comprises a feed additive carrier (i.e., one or more feed additive carriers).
- [0300] Examples of suitable feed additive carriers include, but are not limited to, plant materials (i.e., whole plants or plant parts (e.g., seeds, stems, leaves, flowers, and/or roots, for example), including dried or processed plants or plant parts), dried grains (e.g., distillers' dried grains), alfalfa, corn meal, citrus meal, fermentation residues, ground oyster shells, attapulgius clay, wheat shorts, molasses solubles, corn cob meal, edible vegetable substances, toasted dehulled soya flour, soybean mill feed, antibiotic mycelis, vermiculite, soya grits, whey, maltodextrin, sucrose, dextrose, limestone (calcium carbonate), rice hulls, yeast culture, dried starch, sodium silica aluminate, water, salt solutions, alcohol, silicone, waxes, petroleum jelly, vegetable oils, polyethylene glycols, propylene glycol, liposomes, sugars, gelatin, lactose, amylose, magnesium stearate, talc, surfactants, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethyl-cellulose, polyvinylpyrrolidone, and the like, and combinations thereof.
- [0301] In some aspects, the feed additive comprises an excipient (i.e., one or more excipients) including, but not limited to, microcrystalline cellulose; lactose; sodium citrate; calcium carbonate; dibasic calcium phosphate and glycine; disintegrants such as starch, sodium starch glycollate, croscarmellose sodium and certain complex silicates; granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), sucrose, gelatin, and acacia; bulking agents such as maltodextrin; moisture scavengers such as silicon dioxide; oxygen scavengers such as ascorbic acid; and/or lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate, and talc.
- [0302] In some aspects, the feed additive is a granule comprising: a core comprising the anaerobic cells and/or *M. elsdenii* cells and/or feed additive, and a coating over the core. In some aspects, the coating is a hydrated barrier salt. The salt coating can provide improved thermo-tolerance, improved storage stability, and protection against other components in the granules that may otherwise have adverse effect (e.g., on stability) of the *M. elsdenii* cells and/or feed additive.
- [0303] In some aspects, the encapsulated anaerobic cells and/or *M. elsdenii* cells are admixed with a dry formulation of additives including, but not limited to, growth

substrates, enzymes, sugars, carbohydrates, extracts, and growth promoting micro-ingredients. The sugars can include, but are not limited to, lactose, maltose, dextrose, maltodextrin, sucrose, glucose, fructose, mannose, tagatose, sorbose, raffinose, amylose, starch, and galactose. The sugars can range from 50-95%, either individually or in combination. The extracts can include, but are not limited to, yeast or dried yeast fermentation solubles ranging from 5-50%. The growth substrates can include, but are not limited to, trypticase, ranging from 5-25%; sodium lactate, ranging from 5-30%; and Tween 80, ranging from 1-5%. The carbohydrates can include, but are not limited to, mannitol, sorbitol, adonitol and arabitol. The carbohydrates can range from 5-50% individually or in combination. The micro-ingredients can include, but are not limited to, calcium carbonate, ranging from 0.5-5.0%; calcium chloride, ranging from 0.5-5.0%; dipotassium phosphate, ranging from 0.5-5.0%; calcium phosphate, ranging from 0.5-5.0%; manganese proteinate, ranging from 0.25-1.00%; and manganese, ranging from 0.25-1.00%.

[0304] In some aspects, the anaerobic cells and/or *M. elsdenii* feed additive is prepared by mixing (e.g., with a mixer) anaerobic cells and/or *M. elsdenii* cells, including a culture comprising the cells and/or encapsulated cells, with any additional components of the feed additive (e.g., a carrier and/or an excipient). In some aspects, the components are mixed to obtain a uniform mixture.

[0305] In some aspects, the feed additive is a top-dress animal feed additive comprising anaerobic cells and/or *M. elsdenii* cells as disclosed herein (e.g., encapsulated cells) and a carrier. In some aspects, the carrier is selected from the group consisting of: whey, maltodextrin, sucrose, dextrose, limestone (i.e., calcium carbonate), rice hulls, yeast culture, dried starch, and sodium silica aluminate, milk, water, and combinations thereof.

[0306] In some aspects, the animal feed additive is a drench, spray, or supplement of a milk replacer comprising anaerobic cells and/or *M. elsdenii* cells as disclosed herein (e.g., encapsulated cells) and a water soluble carrier. In some aspects, the carrier is selected from the group consisting of: whey, maltodextrin, sucrose, dextrose, dried starch, sodium silica aluminate, milk, water, and combinations thereof.

[0307] In some aspects, the present invention is directed to a food (e.g., an animal feed) comprising anaerobic cells and/or *M. elsdenii* cells (e.g., encapsulated cells as disclosed herein, e.g., encapsulated cells produced by a method as disclosed herein) and/or a feed

additive as disclosed herein. A food product is any food for animal consumption (i.e., non-human animals or humans), and includes both solid and liquid compositions. Foods include, but are not limited to, common foods; liquid products, including waters, milks, beverages, therapeutic drinks, and nutritional drinks; functional foods; supplements; nutraceuticals; infant (i.e., including non-human and human infants) formulas, including formulas for pre-mature infants; foods for pregnant or nursing animals; foods for adult animals; and geriatric foods. In some aspects, the food includes a liquid (e.g., a drink, e.g., water, milk, or a milk replacer) comprising the feed additive.

[0308] In some aspects, the present disclosure is directed to a composition comprising anaerobic cells and/or *M. elsdenii* cells (e.g., encapsulated cells) and/or a feed additive as disclosed herein. In some aspects, the composition comprises encapsulated anaerobic cells and/or *M. elsdenii* cells produced by a method disclosed herein.

[0309] A composition of the disclosure can include one or more excipients. In some aspects, the excipient can be, but is not limited to, an alkaline agent, a stabilizer, an antioxidant, an adhesion agent, a separating agent, a coating agent, an exterior phase component, a controlled-release component, a solvent, a surfactant, a humectant, a buffering agent, a filler, an emollient, or combinations thereof. Excipients in addition to those discussed herein can include excipients listed in, though not limited to, *Remington: The Science and Practice of Pharmacy*, 21st ed. (2005). Inclusion of an excipient in a particular classification herein (e.g., "solvent") is intended to illustrate rather than limit the role of the excipient. A particular excipient can fall within multiple classifications.

[0310] In some aspects, the composition is a pharmaceutical composition (e.g., for treatment of non-human animals or humans). In some aspects, the composition is a medical food (e.g., a veterinary food). A medical food includes a food that is in a composition to be consumed or administered externally under the supervision of a doctor (e.g., a veterinarian) and that is intended for the specific dietary management of a condition, for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation. In some aspects, the pharmaceutical composition comprises a pharmaceutically acceptable excipient. In some aspects, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized international pharmacopeia for use in animals, and more particularly in humans.

[0311] For oral administration of a composition, the anaerobic cells and/or *M. elsdenii* cells (e.g., encapsulated cells) or feed additive can be combined with excipients well known in the art. Such carriers can, for example, allow the anaerobic cells and/or *M. elsdenii* cells or feed additive of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. In some aspects, the composition is a tablet, pill, caplet, or capsule. Suitable excipients include, but are not limited to, fillers such as sugars, including, but not limited to, lactose, sucrose, mannitol, and sorbitol; cellulose preparations such as, but not limited to, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl cellulose, sodium carboxymethyl cellulose, and polyvinylpyrrolidone (PVP). If desired, disintegrating agents can be added, such as, but not limited to, the cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Compositions that can be used orally include, but are not limited to, capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. In some aspects, the dosage form is a vegetarian dosage form, in which the dosage form is not formed from and does not contain any components from an animal source. In some aspects, the vegetarian dosage form is a vegetarian capsule.

[0312] In some aspects, the present invention is directed to kits or packages comprising anaerobic cells and/or *M. elsdenii* cells, feed additives, foods, and/or compositions as disclosed herein. Kits or packages can include units of a feed additive, food, composition, or combinations thereof (e.g., one or more units). In some aspects, the kit comprises encapsulated freeze-dried cells produced by a method disclosed herein, a feed additive as disclosed herein, or a capsule as disclosed herein.

Methods of Administering Encapsulated Anaerobic Bacterial Cells and/or *Megasphaera elsdenii* to Animals

[0313] In some aspects, provided herein is a method for treating or preventing a condition or disorder associated with lactic acid production in the gastrointestinal tract of a subject, comprising administering to the subject an effective amount of any of the compositions comprising encapsulated *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state disclosed herein, feed additive compositions disclosed herein or any of the feeds disclosed herein.

- [0314] In some aspects, the condition or disorder is acidosis.
- [0315] In some aspects, the condition or disorder is ruminal acidosis.
- [0316] In some aspects, the condition or disorder is respiratory disease.
- [0317] In some aspects, the condition or disorder is laminitis.
- [0318] In some aspects, provided herein is a method for preventing or decreasing the growth of an opportunistic microorganism in the gastrointestinal tract of an animal, comprising administering to the animal an effective amount of any of the compositions comprising encapsulated *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state disclosed herein, feed additive compositions disclosed herein or any of the feeds disclosed herein.
- [0319] In some aspects, the opportunistic microorganism is pathogenic.
- [0320] In some aspects, the opportunistic microorganism is *Salmonella*, *Escherichia Coli*, or *Campylobacter*.
- [0321] In some aspects, provided herein is a method for improving the growth performance of a subject comprising administering to the subject an effective amount any of the compositions comprising encapsulated *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state disclosed herein, feed additive compositions disclosed herein or any of the feeds disclosed herein. In some aspects, improving the performance of a subject comprises of one or more of feed conversion ratio (FCR), weight gain, feed efficiency, carcass quality, reducing mortality, reducing morbidity, feed intake, average daily gain, carcass gain, bone mineralization, egg production, reduced digestive tract dysbiosis or dysfunction, milk composition (e.g., increased milk fat), and/or milk production compared to the performance of a subject that has not been administered the feed additive composition or feed.
- [0322] In some aspects, provided herein is a method for increasing starch digestibility, lowering fecal starch output, and/or preventing a decrease in the pH in the lower gastrointestinal tract in a subject comprising adding an effective amount of any of the compositions comprising encapsulated *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state disclosed herein, feed additive compositions disclosed herein to a feed for administration to a subject, wherein the subject exhibits one or more of increased starch digestibility and/or lowered fecal starch

output compared to a subject that has not been administered the compositions, feed additive composition, or feeds.

- [0323] In some aspects, provided herein is a method for increasing operational efficiency of a farm, comprising administering to an animal on the farm an effective amount of any of the compositions comprising encapsulated *Megasphaera elsdenii* cells, anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state disclosed herein feed additive compositions disclosed herein or any of the feeds disclosed herein. In some aspects, the increased operational efficiency results decreased labor costs, decreased roughage transport costs, and decreased amount of roughage added to a feed or feed additive.
- [0324] In some aspects, the subject is a ruminant.
- [0325] In some aspects, the ruminant is selected from the group consisting of cattle, goats, sheep, giraffes, deer, gazelles, buffalo, reindeer, and antelopes.
- [0326] In some aspects, the cattle are beef cattle or dairy cattle.
- [0327] In some aspects, the subject is a non-ruminant.
- [0328] In some aspects, the non-ruminant is selected from the group consisting of: equine, poultry, and swine.
- [0329] In some aspects, the poultry is selected from the group consisting of: a chicken, a goose, a duck, a quail, a turkey, broiler, broiler breeder, a layer, or a pigeon.
- [0330] In some aspects, the poultry is a chicken.
- [0331] In some aspects, the feed additive composition or feed is provided to the subject for daily administration or for weekly administration.
- [0332] In some aspects, the method comprises administering to the animal encapsulated *M. elsdenii* cells, a feed additive, a food, or a composition (e.g., a capsule) as described herein.
- [0333] In some aspects, the present invention is directed to a method of administering encapsulated anaerobic bacterial cells to an animal. In some aspects, the present invention is directed to a method of administering encapsulated *Bifidobacterium* cells, such as *B. breve*, *Lactobacillus* cells, such as *L. plantarum*, *Bifidobacterium* cells, such as *B. animalis* subsp. *lactis*, *Pediococcus* cells, such as *P. acidilactici*, *Lactobacillus* cells, such as *L. casei* cells, *Fibrobacter*, such as *F. succinogenes* cells, *Butyrivibrio*, such as *B. fibrisolvens* cells, *Ruminococcus* cells, such as *R. flavefaciens*, *Blautia* cells, such as *B.*

obeum, *Clostridium* cells, such as *C. butyricum*, *Akkermansia* cells, such as *A. muciniphila* and *Megasphaera* cells, such as *M. elsdenii*, to an animal.

[0334] The administration can be by any compatible route, including, for example, orally (i.e., an ingestible liquid or solid, an oral drench, a feed additive, a food, a composition, or a capsule), by spraying onto the body (i.e., by mist spraying), and/or injection.

[0335] In some aspects, the method comprises administering a solid, a liquid, or a gel comprising the encapsulated *M. elsdenii* cells or anaerobic bacterial cells.

[0336] In some aspects, the method comprises administering a solid feed additive comprising the encapsulated *M. elsdenii* cells or anaerobic bacterial cells. In some aspects, the solid feed additive is a powder (e.g., a flowable powder), granule (i.e., a granulate), particle (i.e., particulate), pellet, cake, water soluble concentrate, paste, bolus, tablet, dust, a component thereof, or combinations thereof.

[0337] In some aspects, the method comprises administering a liquid feed additive comprising the encapsulated *M. elsdenii* cells or anaerobic bacterial cells. In some aspects, the method comprises administering the encapsulated *M. elsdenii* cells or anaerobic bacterial cells in a liquid. In some aspects, the liquid is a solution (e.g., an aqueous, organic, or aqueous-organic solution), suspension, emulsion, drench, spray, injectable, drink (e.g., a milk replacer), a component thereof, or combinations thereof. In some aspects, the liquid is administered orally or by spraying the animal with the liquid.

[0338] In some aspects, the method comprises combining the encapsulated *M. elsdenii* cells or anaerobic bacterial cells or the feed additive comprising the cells with another animal feed additive to form a supplement or premix for adding to an animal feed. In some aspects, the other feed additive comprises cells other than *M. elsdenii* cells.

[0339] In some aspects, the encapsulated *M. elsdenii* cells or anaerobic bacterial cells can be added to the feed additive as a liquid (e.g., in a broth or broth equivalent, including, e.g., rehydrated electrospray-dried cells), or as a reconstituted cell paste. Dosage forms (e.g. drench of predetermined volume or capsules) can also be formed and, if desired, the encapsulated *M. elsdenii* or anaerobic cells can be added directly to the animal feed, as by sprinkling a liquid broth and/or encapsulated *M. elsdenii* or anaerobic cells over the feed or mixing into the feed.

- [0340] In some aspects, the method comprises rehydrating a feed additive (e.g., a powder, granulate, particulate, pellet, cake, electrospray-dried cells, or combinations thereof) to produce a liquid for administration.
- [0341] In some aspects, the method comprises applying encapsulated *M. elsdenii* cells or anaerobic bacterial cells to animal feed through a delivery system that rehydrates a feed additive, including on a batch-to batch basis. For example, an electrospray dried powder can be augured from a polyvinyl hopper into a flushing system, which dilutes the powder and sprays it on the feed to be mixed.
- [0342] In some aspects, the method comprises applying encapsulated *M. elsdenii* cells or anaerobic bacterial cells to animal feed using a volumetric metering device with a storage bin. For example, the encapsulated *M. elsdenii* cells or anaerobic bacterial cells (e.g., a powder comprising the cells) can be stored in the storage bin and discharged into a water or aqueous bath just prior to being sprayed onto an animal feed.
- [0343] In some aspects, the present invention is directed to a method for treating or preventing a condition or disorder associated with lactic acid production in the gastrointestinal tract of an animal, comprising administering to the animal an effective amount of encapsulated *M. elsdenii* cells as disclosed herein, encapsulated *M. elsdenii* cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition (e.g., a capsule) as disclosed herein.
- [0344] In some aspects, the condition or disorder is acidosis. In some aspects, the condition or disorder is ruminal acidosis. In some aspects, the condition or disorder is respiratory disease. In some aspects, the condition or disorder is laminitis. In some aspects, the condition or disorder is an infection. In some aspects, the infection is with *Salmonella* or *Campylobacter*. In some aspects, the *Salmonella* is *Salmonella enterica* and/or *Salmonella bongori*. In some aspects, the *Salmonella* serotype is *Salmonella Typhimurium* and/or *Enteritidis*. In some aspects, the *Campylobacter* is *Campylobacter jejuni* or *Campylobacter coli*.
- [0345] In some aspects, the present invention is directed to a method for preventing or decreasing the growth of an opportunistic microorganism in the gastrointestinal tract of an animal, comprising administering to the animal an effective amount of encapsulated *M. elsdenii* cells as disclosed herein, encapsulated *M. elsdenii* cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein.

- [0346] In some aspects, the present invention is directed to a method for preventing or decreasing the growth of an opportunistic microorganism in the gastrointestinal tract of an animal, comprising administering to the animal an effective amount of encapsulated anaerobic bacterial cells as disclosed herein, encapsulated anaerobic cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein.
- [0347] In some aspects, the opportunistic microorganism is pathogenic. In some aspects, the opportunistic microorganism is *Salmonella* or *Campylobacter*. In some aspects, the *Salmonella* is *Salmonella enterica* and/or *Salmonella bongori*. In some aspects, the *Salmonella* serotype is *Salmonella Typhimurium* and/or *Enteritidis*. In some aspects, the *Campylobacter* is *Campylobacter jejuni* or *Campylobacter coli*.
- [0348] In some aspects, the present invention is directed to a method of improving the bioavailability of plant-derived phosphorous in the diet of an animal, comprising administering to the animal an effective amount of encapsulated *M. elsdenii* cells as disclosed herein, encapsulated *M. elsdenii* cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein. In some aspects, the encapsulated *M. elsdenii* cells comprise a phytase activity. In some aspects, the method reduces environmental phosphorous waste resulting from administration of an animal diet in the absence of *M. elsdenii* cells.
- [0349] In some aspects, the present invention is directed to a method of improving the bioavailability of plant-derived phosphorous in the diet of an animal, comprising administering to the animal an effective amount of encapsulated anaerobic bacterial cells as disclosed herein, electrospray dried anaerobic bacterial cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein. In some aspects, the encapsulated anaerobic bacterial cells comprise a phytase activity. In some aspects, the method reduces environmental phosphorous waste resulting from administration of an animal diet in the absence of the anaerobic bacterial cells.
- [0350] In some aspects, the present invention is directed to a method of improving growth performance in an animal, comprising administering to the animal an effective amount of encapsulated *M. elsdenii* cells as disclosed herein, encapsulated *M. elsdenii* cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein.

- [0351] In some aspects, the present invention is directed to a method of improving growth performance in an animal, comprising administering to the animal an effective amount of encapsulated anaerobic bacterial cells as disclosed herein, encapsulated anaerobic bacterial cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein.
- [0352] In some aspects, the improved growth performance in the animal is an improvement in: feed intake, average daily gain, feed conversion ratio, carcass gain, milk composition in a milk-producing animal (e.g., increased milk fat), milk production in a milk-producing animal, egg production in poultry, bone mineralization, or combinations thereof.
- [0353] In some aspects, the present invention is directed to a method of acidifying the lower gastrointestinal tract of an animal, comprising administering to the animal an effective amount of encapsulated *M. elsdenii* cells as disclosed herein, encapsulated *M. elsdenii* cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein. In some aspects, the lower gastrointestinal tract is the ceca of a poultry animal.
- [0354] In some aspects, the present invention is directed to a method of acidifying the lower gastrointestinal tract of an animal, comprising administering to the animal an effective amount of encapsulated anaerobic bacterial cells as disclosed herein, encapsulated anaerobic bacterial cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein. In some aspects, the lower gastrointestinal tract is the ceca of a poultry animal.
- [0355] In some aspects, the encapsulated *M. elsdenii* cells or anaerobic bacterial cells as disclosed herein, encapsulated *M. elsdenii* cells or anaerobic bacterial cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein is administered prior to, concomitantly with, or after feeding the animal with a food.
- [0356] In some aspects, the method further comprises mixing encapsulated *M. elsdenii* cells or anaerobic bacterial cells as disclosed herein, the encapsulated *M. elsdenii* cells or anaerobic bacterial cells produced by a method as disclosed herein, or a solid feed additive as disclosed herein with a liquid prior to administration.

- [0357] In some aspects, a liquid is administered orally (e.g., an oral drench) or by spraying (e.g., mist spraying) the animal with the liquid.
- [0358] In some aspects, the method comprises a single administration of encapsulated *M. elsdenii* cells or anaerobic bacterial cells as disclosed herein, encapsulated *M. elsdenii* cells or anaerobic bacterial cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein.
- [0359] In some aspects, the method comprises a daily administration of encapsulated *M. elsdenii* cells or anaerobic bacterial cells as disclosed herein, encapsulated *M. elsdenii* cells or anaerobic bacterial cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein. In some aspects, the administration is at least once daily, at least twice daily, at least three times daily, or more than three times daily. In some aspects, the administration is *ad libitum* (e.g., self-administration by drinking an available liquid or eating an available food comprising the encapsulated *M. elsdenii* or anaerobic cells, the encapsulated *M. elsdenii* or anaerobic cells produced by a method as disclosed herein, the feed additive, or the composition).
- [0360] In some aspects, the method comprises more than one administration on a single day of encapsulated *M. elsdenii* cells or anaerobic bacterial cells as disclosed herein, encapsulated *M. elsdenii* cells or anaerobic bacterial cells produced by a method as disclosed herein, a feed additive as disclosed herein, or a composition as disclosed herein. In some aspects, the administration is two, three, four, five, six, or more administrations on a single day. In some aspects, the method comprises more than one administration on a single day followed by one or more days without administration. In some aspects, the one or more days without administration is one, two, three, four, five, or six days, one week, two weeks, three weeks, or four weeks, one month, two months, three months, four months, five months, or six months without administration.
- [0361] In some aspects, the animal is a ruminant. In some aspects, the ruminant can be, but is not limited to, cattle, buffalo, sheep, goats, deer, reindeer, moose, giraffe, yaks, and elk. In some aspects, the ruminant is selected from the group consisting of: cattle, buffalo, sheep, goats, deer, and reindeer.
- [0362] In some aspects, the animal is a non-ruminant. In some aspects, the non-ruminant can be, but is not limited to, equines, poultry, swine, dogs, humans, and cats. In some

aspects, the non-ruminant is selected from the group consisting of: equines, poultry, and swine.

[0363] In some aspects, the animal is a zoo animal.

[0364] In some aspects, the animal is a poultry animal. In some aspects, the poultry animal is an avian (i.e., bird) that is used as a food animal including, but not limited to, a chicken, goose, duck, quail, turkey, pigeon, emu, or ostrich. In some aspects, the poultry animal is selected from the group consisting of: a chicken, a goose, a duck, a quail, a turkey, or a pigeon. In some aspects, the poultry animal is selected from the group consisting of: a broiler, a broiler breeder, and a layer. In some aspects, the poultry animal is a chicken.

[0365] In some aspects, the animal is an equine. In some aspects, the equine is a horse, a pony, a donkey, or a mule.

[0366] In some aspects, the animal is a companion animal. In some aspects, the companion animal is a dog, cat, guinea pig, rabbit, rat, mouse, or horse. In some aspects, the companion animal is a dog. In some aspects, the animal is a cat.

EXAMPLES

[0367] Reference is now made to the following examples, which together with the above descriptions illustrate some aspects of the invention in a non-limiting fashion.

Example 1: Formulation assessment

[0368] *M. elsdenii* is a strict anaerobic bacterium with high potential for use as a direct fed microbial in the cattle industry. However, due to their anaerobic nature, *M. elsdenii* have proved difficult to stabilize and deliver in feed at commercial feedlot/dairy facilities. Similar difficulties can occur with anaerobic bacteria that may be used at commercial feedlot/dairy facilities.

[0369] In the examples below, different types of encapsulations were tested on freeze dried culture of *M. elsdenii* NCIMB 41125. All *M. elsdenii* cultures were grown, cooled, concentrated, and freeze-dried (FD) following method described in WO 2018/144653 A1, which is incorporated by reference herein. The resulting freeze-dried *M. elsdenii* cultures were used to prepare the test formulations and were then assessed by:

- recovery post-encapsulation – to determine commercial feasibility.

- recovery post-environmental exposure in micromachine bins – to determine the impact of environmental exposure prior to inclusion in the feed via the micromachine.
- survival through the micromachine – to determine the level of protection provided to *M. elsdenii* after passing through the micromachine, which are commonly used at commercial feedlot to dispense "small" ingredients into feed.
- rumen release – to determine *M. elsdenii* release in the rumen.
- survival when mixed in feed – to determine the level of protection provided to *M. elsdenii* through the in-feed mixing, but prior to ingestion by the animal.
- stability data (shelf life) – to determine *M. elsdenii* stability in formulations stored for extended period of time at various temperatures.

[0370] The following test protocols were used for all formulations unless specified otherwise:

[0371] Recovery post-encapsulation: Encapsulated products (formulations) were analyzed for *M. elsdenii* colony forming unit (CFU) and compared to initial *M. elsdenii* freeze-dried culture concentration to determine percent recovery post encapsulation.

[0372] Briefly, encapsulated material (formulation) was blended into rehydrant under anaerobic conditions at room temperature. After which samples were plated in triplicates on semi-defined lactate (SDL) agar plates and incubated for 48 hours at 39° C in an anaerobic chamber. CFU results were then adjusted for amount of freeze-dried culture in different formulations and expressed as CFU/g of freeze-dried culture. Percent recovery post encapsulation was calculated by dividing the CFU/g post encapsulation by the CFU/g pre-encapsulation.

[0373] Recovery post environmental exposure in micromachine bins: Low inclusion feed ingredients (i.e., < 500 mg for direct fed microbials) were precisely added to the feed batch/load at commercial feedyards and dairies using micromachines. Prior to addition to the micromachines, micro-ingredients were stored in individual bins connected to a scale and computerized management system. Micro-ingredients can stay for an extensive period, up to 14 hours, in the micromachine's bins before being mixed in the feed. To determine the loss incurred during environmental exposure in the micromachine bins, formulations were incubated at 25, 30, and 37° C for up to 14 hours. This experiment was repeated three times.

- [0374] Formulations aliquots (20 g) were weighed in weight boats and placed in incubators set at 25, 30, or 37° C. After the appropriate incubation time, weight boats were retrieved and transferred to the humidity control chamber to be further divided into 1.5 g aliquots. Those aliquots were rehydrated in the anaerobic chamber in rehydrant and blended. After blending, all samples were allowed to rehydrate for 2 hours prior to being diluted and plated onto semi-defined lactate agar. CFU results were expressed as CFU/g of formulations. Percent recovery post-incubation in the micromachine bin was calculated by dividing the CFU/g post-incubation by the CFU/g pre-incubation.
- [0375] Survival through the micromachine: Micro-ingredient inclusion weights were based on product dose, quantity of feed/batch, and number of animals fed/batch of feed. The micromachines were calibrated to the expectations of the appropriate product amount with allowed tolerances. The computer pre-weighs ingredients, which were dropped from the bins and put into suspension in a pool of water. The mixture was then flushed with water, which served as a carrier to deliver the micro-ingredients to the finished feed batch. The micro ingredient/water mixture was applied to the feed in the feed truck using a spray bar and is then thoroughly mixed into the feed.
- [0376] Two water administration methods were tested.
- [0377] (1) Bowl system: Micro-ingredients were measured into a hopper that dumps into a slurry mixing bowl. The slurry contents were mixed with water using impellers. The mix was flushed from the mixing tank with water to ensure complete emptying of the tank. A pump moved the ingredient/water mixture to the spray bar.
- [0378] (2) Continuous flow system: Micro-ingredients were weighed and dispensed into a continuous flow of water that delivers the ingredients/water mixture to the spray bar. This system produced a higher inclusion rate of ingredients and can be used for larger batch size.
- [0379] Prior to starting the experiment, the system was flushed out to ensure no additional debris were dispensed into the sample. After flushing, a funnel with a mesh bag was placed inside the sprayer's drain and a collection bucket was placed underneath to collect the sample and water being dispensed.
- [0380] A known amount of product (formulation) was slowly added to the micromachine and water was flushed through the system until all product dispensed. Run time was

recorded and the system was left running for an additional 15-30 seconds to clear any remaining product in the micromachine and hose.

[0381] Collected samples were transferred into the anaerobic chamber, rehydrated, and blended. After blending, all samples were allowed to rehydrate at room temperature for 2 hours before being diluted and plated onto SDL agar plates. Plates were incubated for 48 hours at 39°C under anaerobic conditions.

[0382] CFU results were then adjusted for amount of product and expressed as CFU/g of product (formulation). Percent recovery after micromachine was calculated compared to concentration of encapsulated product prior to passage through micromachine (CFU/g).

[0383] In some examples, resulting samples were mixed in feed after passage through the micromachine to mimic feedlot application, following the described infeed survival protocol. CFU results were then adjusted for amount of product and expressed as CFU/g of product (formulation). Percent recovery after incubation in the feed (diet) was calculated compared to concentration of encapsulated product prior to mixing in feed.

[0384] Rumen release: Four fistulated steers were used to determine release of the formulations *in vivo*. Two different diets were fed to the animals (2 steers/diet): high forage (80% roughage and 20% concentrate) and high concentrate (20% roughage and 80% concentrate).

[0385] Empty Dacron bags were labelled, dried, and weighed prior to being filled with encapsulated product (formulations). Bags were weighed again (initial weight) and heat sealed. Dacron bags were then placed in a large nylon-mesh bag with a weight to be incubated/suspending in the ventral sac of the rumen for a total of 24 hours (1 bag per animal; 2 dacron bags per treatment per bag per animal). Two empty Dacron bags per steer were also included as blanks.

[0386] On removal from the steers, the nylon-mesh bags were rinsed and dried for 72 hours in an incubator set to 55° C. After drying, bags were weighed to determine final weight. Ruminant dry matter (DM) disappearance was then calculated as follow:

[0387]
$$\% \text{ Ruminant Dry Matter Disappearance} = \frac{\text{Initial weight} - \text{Final weight}}{(\text{Initial weight} - \text{Empty bag weight}) \times (\text{non lipid content})}$$

[0388] The lipid material present in the encapsulation should not be degraded over the 24-hour period, and therefore, the initial weight was corrected for its non-lipid content. For the purpose of these Examples, the amount of dry matter disappearing in the rumen

was considered as the amount of dry matter being released/made available to the animal (rumen release).

[0389] *In feed survival:* The following representative feedlot diets were prepared and placed into individual container (250-mL capacity) in 30 gram aliquots.

Table 2. Low pH and high moisture diet composition - 4.3 pH, 40% Moisture.

Ingredients	% of diet, as-is
Steam-flaked corn	46.4
Corn silage	27.2
Wet corn gluten feed	24.4
Supplement (minerals, e.g., Fe, Zn, and/or Mn)	2.0
Total	100.0

Table 3. High pH and high moisture diet composition— 5.6 pH and 43.6% moisture.

Ingredients	% of diet, as-is
Steam Flaked Corn	59.23
Ground Alfalfa Hay	5.52
Concentrated Separator By-Product	9.09
Supplement	3.33
RO Water	22.83
Total	100.0

Table 4. High pH and low moisture diet composition – 5.8 pH and 9% moisture.

Ingredients	% of diet, as-is
Steam Flaked Corn	82.5
Sorghum Silage	6.7
Alfalfa	3.0
Supplement	6.8
Feed Additive	1.0
Total	100.0

[0390] A sample from each formulation was weighed and placed on top of the diet in a container. Each container was individually mixed by hand and either placed on the bench top at room temperature (25° C) or incubated at 52° C under aerobic conditions. Containers were sampled at the designated timepoint.

[0391] After 0, 2, and 4 hours, designated containers were brought into the anaerobic chamber, their contents were rehydrated, blended, and left to rehydrate at room

temperature for 2 hours. A 1 mL sample was then collected from each container, diluted, and plated onto SDL agar plates. Plates were incubated for 48 hours at 39° C under anaerobic conditions.

[0392] CFU results were then adjusted for amount of freeze-dried powder in different formulations and expressed as CFU/g of freeze-dried culture. Percent recovery post-mixing (time 0) and after 2 or 4 hours incubation in the diet (time 2 or 4) were calculated compared to concentration of formulations prior to mixing in the diet (CFU/g).

[0393] Stability/Shelf life: Stability of the formulations were tested in bulk by aliquoting samples into large resealable Mylar Foil bags (12"x12"), or in individual bags, by aliquoting samples into small Mylar Foil bags (one time use). Bags were properly labelled and stored at either -20° C or 4° C to be sampled after 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, and 12 months. Three bags per formulation, per temperature, and per time point.

[0394] On sampling day, samples (1.5 g; individual bag content or aliquot of bulk bag) were weighed and transferred into the anaerobic chamber. Samples were then rehydrated, blended, and allowed to rehydrate for 10 minutes before being plated on SDL agar. After a 48-hour incubation period at 39° C the plates were counted, and results recorded as CFU/g of formulation. Stability data were plotted against time.

Example 2: Influence of carrier in cell recovery post-encapsulation and stability during storage at -20° C or 4° C.

[0395] Experiments were performed to determine the effect of different carriers on *M. elsdenii* cell recovery post-encapsulation and stability during storage.

[0396] Freeze dried *M. elsdenii* cultures obtained following method described in WO 2018/144653 A1 were mixed with one of three different types of starch – potato, wheat, or corn – to form a matrix and were then encapsulated with vegetable oil (a mixture of mono- and di-glycerides of palmitic, stearic, oleic, linoleic, and linolenic acids) at a 40/60 ratio (40% matrix and 60% coating) at 52° C (Table 5) in a Wurster-type fluid bed agglomerating coater.

[0397] Cell recovery (Table 5) was tested following the protocol described in **Example 1** and samples were bulk packaged to perform stability study at either -20 or 4° C. Samples were analyzed after 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, and 12 months of storage (Figure 1) following protocol described in **Example 1**.

Table 5. Formulation composition and cell recovery post-encapsulation with different types of starch as bulking agents.

Portion	Ingredient*	Potato starch (PS)	Wheat Starch (WS)	Corn Starch (CS)
Matrix	FD <i>M. elsdenii</i>	1.89	1.89	1.89
	Carrier	38.11	38.11	38.11
	Total Matrix	40.00	40.00	40.00
Coat	Vegetable oil [@]	60.00	60.00	60.00
	Total Lipid	60.00	60.00	60.00
	CFU/g of Matrix	3.50 x 10 ⁹	2.79 x 10 ⁹	2.49 x 10 ⁹
	% Recovery^s	71.53	61.22	52.71
*Ingredients expressed as % of total. [@] mixture of mono- and di-glycerides of palmitic, stearic, oleic, linoleic, and linolenic acids. ^s Percent recovery calculated based on CFU/g of Matrix				

[0398] Formulations with corn and wheat starches, as a carrier, had lower cell recovery through the encapsulation process than the formulation containing potato starch.

[0399] Storage temperatures did not affect cell loss during the 12-month period. Most of the loss observed during shelf life occurred early in the storage period (within the first 15 days) regardless of the treatments. Loss over the 12-month storage period was consistent regardless of the type of starch used as a carrier pre-encapsulation, ranging from 0.4 to 0.6 log CFU/g. See Figure 1.

[0400] Similar, or greater cell recovery through the encapsulation process is expected when performing a two-step encapsulation as described in **Example 19**.

[0401] Likewise, the storage temperature is not expected to affect cell loss during the 12-month storage period regardless of the type of starch used as a carrier prior to a two-step encapsulation as described in **Example 19**.

Example 3: Vegetable oil encapsulation provides cell protection during exposure to low pH and high moisture feed.

[0402] To determine the level of protection provided to *M. elsdenii* by a vegetable oil encapsulation, the encapsulated cells were mixed in a high moisture and low pH feedlot diet at room temperature (25° C) for up to 4-hours.

[0403] Freeze dried *M. elsdenii* culture was obtained following method described in WO 2018/144653 A1. The resulting culture was mixed with potato starch, as a carrier, to form a matrix and was then encapsulated with vegetable oil (a mixture of mono- and di-glycerides of palmitic, stearic, oleic, linoleic, and linolenic acids) at a 40/60 ratio (40%

matrix and 60% vegetable oil) at 52° C in a Wurster-type fluid bed agglomerating coater. The resulting formulation (vegetable oil (VO) formulation) was rapidly cooled to maximize cell recovery.

[0404] Cell recovery (Table 6) post-encapsulation was tested following the protocol described in **Example 1**. Freeze dried culture, matrix, and vegetable oil (VO) formulation were mixed with a low pH and high moisture feedlot diet (Table 2) for 4 hours at room temperature, and used in the in feed survival protocol described in **Example 1** and Table 7.

Table 6. Sample composition

Portion	Ingredient*	FD <i>M. elsdenii</i>	Matrix	VO Formulation
Matrix	FD <i>M. elsdenii</i>	100	4.72	1.89
	Potato Starch	-	95.28	38.11
	Total Matrix		100.00	40.00
Coat	Vegetable oil@	-	-	60.00
	Total Lipid	0	0	60.00
% Recovery				33.30
*Ingredients expressed as % of total.				
@mixture of mono- and di-glycerides of palmitic, stearic, oleic, linoleic, and linolenic acids.				

Table 7. In feed survival testing - Content description and rehydration details.

Treatment	Sample, g	Diet, g	Rehydrant, mL	Blending
FD <i>M. elsdenii</i>	0.16	-	20 mL	Hand agitation
FD <i>M. elsdenii</i> + Diet*	0.5	30	270 mL	2 min blend
Matrix	2.5	-	22.5 mL	Hand agitation
Matrix + Diet*	0.5	30	270 mL	2 min blend
VO Formulation	0.03	-	300 mL	3 min blend
VO Formulation + Diet*	2.5	30	270 mL	2 min blend
*Low pH, high moisture diet.				

[0405] *M. elsdenii* recovery post-in feed mixing (T0) and after 4-hour exposure (T4) at 25° C was calculated by comparing concentrations obtained to concentration of formulations prior to mixing with the diet (Table 8).

Table 8. *M. elsdenii* recovery after mixing treatments into low pH and high moisture diet for 0 or 4 hours at 25° C under aerobic conditions.

Treatments	% recovery at 25° C	
	0 hours	4 hours
FD <i>M. elsdenii</i>	85.03	7.52
Matrix	12.31	0.02
VO Formulation	103.64	19.56

[0406] Addition of potato starch to FD *M. elsdenii* accelerated decay of *M. elsdenii* when mixed in low pH and high moisture diet compared to FD *M. elsdenii* alone. Survival of *M. elsdenii* was increased with vegetable oil encapsulation (VO formulation) when compared to freeze-dried culture and matrix treatments, but still had low recovery after 4-hour exposure in low pH and high moisture diet, approximately 20%. See Table 8.

Example 4: Effect of fluidizing time and temperature on *M. elsdenii* cell survival.

[0407] To determine optimal temperature during the fluidizing process by testing varying temperatures and fluidizing times, and comparing *M. elsdenii* cell recovery at the end of the process.

[0408] Fluid bed device was preheated to meet target process temperature. The process temperature was set a couple degrees lower than melting point of hydrogenated palm oil (HPO). Once the vessel was at temperature, freeze dried *M. elsdenii* culture was added at the bottom of the vessel and filter pulse intervals were set to 1 second before process began. Airflow was set at 25 liters/minute (LPM) and progressively increased during the fluidizing run up to 160 LPM. Fluidizing was performed for 30 or 60 minutes, after which the device was turned off and the fluidized freeze-dried culture was collected in a sterile sample cup.

[0409] The resulting product was aliquoted into 0.16 g samples inside the humidity control chamber and then transferred to the anaerobic chamber to be rehydrated in 20 mL anaerobic diluent and plated on semi-defined lactate agar. After 48 hours incubation at 39° C, CFU results were used to calculate percent recovery comparing freeze dried culture concentration pre- and post-fluidizing (Table 9).

Table 9. *M. elsdenii* cell recovery following 30 or 60 minutes fluidizing treatment at 50° C, 55° C, 60° C, and 65° C.

Process temperature	30 min fluidizing		60 min fluidizing	
	CFU/g	% recovery	CFU/g	% recovery
50° C	6.42E+10	101.33%	6.48E+10	75.21%
55° C	2.46E+10	38.85%	5.13E+10	59.59%
60° C	1.87E+10	29.52%	1.84E+10	21.32%
65° C	6.17E+09	9.75%	1.11E+10	12.87%

[0410] Cell recovery post-fluidizing (Table 9) was affected by process duration and temperature. The best recovery was obtained after 30 minutes fluidizing at 50° C. *M. elsdenii* cells demonstrated a certain resilience, despite the decrease in recovery, with 10-13% of the cell being able to survive fluidizing process at 65° C for 30-60 minutes.

Example 5: Palm oil encapsulation alone did not provide sufficient moisture and heat protection to *M. elsdenii* when mixed in low pH and high moisture feed.

[0411] Experiments were performed to determine the protection provided by spinning disk encapsulation alone, with an outer coating with a carnauba wax/hydrogenated cottonseed oil mixture sprayed in a Wurster fluid bed agglomerating coater, or with an additional coating layer of carnauba wax.

[0412] *M. elsdenii* culture was grown, cooled, concentrated, and freeze-dried following method described in WO 2018/144653 A1. An aliquot of the resulting freeze-dried culture (3.5 kg) was then encapsulated to form a core containing the freeze-dried (FD) *M. elsdenii* and hydrogenated palm oil (HPO) using spinning disk method. The core was processed to create small or large particles and then spray coated in a Wurster fluid bed agglomerating coater with a mixture of carnauba wax (CW) and hydrogenated cottonseed oil (HCO). In addition, some of the formulations were processed again through the Wurster fluid bed agglomerating coater with carnauba wax to form a second outer coating. Final formulation compositions are listed in Table 10. Melting point of the oils and wax were as follows:

- Hydrogenated Palm Oil = 58° C to 62° C
- Hydrogenated Cottonseed Oil = 60° C to 64° C
- Carnauba wax = 82° C to 86° C

[0413] Encapsulated products were then analyzed for colony forming unit (CFU) and compared to initial freeze-dried powder to determine percent recovery post-encapsulation as described in **Example 1**.

Table 10. Formulation composition and recovery post encapsulation.

Portion	Ingredient*	Small particles (250-1400 μ m)					Large particles (1400-2000 μ m)			
		A	B	C	D	E	F	G	H	I
Core	FD <i>M. elsdenii</i>	30.0	22.5	15.0	11.25	7.5	22.5	15.0	11.25	7.5
	HPO	70.0	52.5	35.0	26.25	17.5	52.5	35.0	26.25	17.5
	Total Core	100.0	75.0	50.0	37.50	25.0	75.0	50.0	37.50	25.0
Coat 1	CW	-	15.0	30.0	22.50	15.0	15.0	30.0	22.50	15.0
	HCO	-	10.0	20.0	15.00	10.0	10.0	20.0	15.00	10.0
Coat 2	CW	-	-	-	25.00	50.0	-	-	25.00	50.0
	Total Coat		25.0	50.0	62.50	75.0	25.0	50.0	62.50	75.0
	Total Lipid	70.0	77.5	85.0	88.75	92.5	77.5	85.0	88.75	92.5
CFU/g		3.0x10 ¹⁰	3.9x10 ¹⁰	4.1x10 ¹⁰	6.1x10 ¹⁰	4.3x10 ¹⁰	5.1x10 ¹⁰	4.9x10 ¹⁰	5.0x10 ¹⁰	4.7x10 ¹⁰
% Recovery ^s		20%	26%	27%	41%	29%	34%	33%	34%	32%

*Ingredients expressed as % of total.

^sRecovery post-encapsulation.

[0414] Formulations containing additional coating around the core survived the encapsulation process better than the one made using HPO and spinning disk method alone. Particle size of the final product did not appear to affect cell recovery post-encapsulation. Overall recovery post-encapsulation was low compared to **Example 2 and 3**.

[0415] Formulations were then tested in feed using a low pH and high moisture diet, representative feedlot diet, prepared as shown in Table 2, to establish in feed survival (Table 11) following the procedure described in **Example 1**.

Table 11. *M. elsdenii* recovery after mixing encapsulated product into low pH and high moisture diet for 0 or 4 hours at 25° C or 52° C under aerobic conditions.

Treatments	% recovery at 25° C		% recovery at 52° C	
	0 hours	4 hours	0 hours	4 hours
A	78%	23%	70%	10%
B	77%	68%	79%	35%
E	89%	83%	76%	20%

[0416] Encapsulation with HPO (core alone; A) yielded low recovery after 4-hour incubation in low pH and high moisture diet at 25 or 52° C (Table 11). This recovery was similar to the one observed with vegetable oil encapsulation (VO Formulation; **Example 3**). Addition of an outer coating, using CW/HCO (B), increased the recovery after 4-hour

exposure by 45% at 25° C and by 25% at 52° C. Addition of another outer coating with CW (**E**), further improved the recovery at 25° C, but yielded lower recovery at 52° C. Recovery observed on freeze dried *M. elsdienii* encapsulated with hydrogenated palm oil (HPO) to form a core and then coated with a mixture of carnauba wax (CW) and hydrogenated cottonseed oil (HCO) with (68%; **B**) or without a second outer coating (83%; **E**) were greater than the recovery previously observed with vegetable oil (**Example 3**; approximately 20%).

Example 6: Two-step encapsulation process is necessary to provide sufficient protection to *M. elsdenii* during exposure to low pH and high moisture feed.

[0417] Next, it was determined if single step encapsulation, spinning disk, or Wurster fluid bed agglomerating coater only could provide sufficient protection to *M. elsdenii* through encapsulation and mixing in feed.

[0418] *M. elsdenii* culture was grown, cooled, concentrated and freeze-dried following method described in WO 2018/144653 A1. An aliquot of the resulting freeze-dried culture was mixed with Maltodextrin at 1:5 ratio and encapsulated as described in **Example 5**. Final formulations compositions are listed in Table 12 & 13. Formulations were produced to have a small final particle size (212-710 µm) to increase surface area and maximize release post ingestion by cattle (rumen release). Encapsulated products were analyzed for colony forming unit (CFU) and compared to initial freeze-dried powder to determine percent recovery post-encapsulation (Table 12 & 13) following protocol described in **Example 1**.

Table 12. Formulations composition and recovery post-encapsulation

Portion	Ingredient*	4	5	17	18	19	20A
Core	FD <i>M. elsdenii</i>	2.5	1.3	13.4	10.9	8.4	5.8
	Maltodextrin	12.5	6.3	66.6	54.1	41.7	29.2
	HPO	85.0	92.5	-	-	-	-
	Total Core	97.5	98.8	80.0	65.0	50.0	35.0
Coat	CW	-	-	6.0	10.5	15.0	19.5
	HCO	-	-	14.0	24.5	35.0	45.5
	Total Coat	0	0	20.0	35.0	50.0	65.0
	Total Lipid	85.0	92.5	20.0	35.0	50.0	65.0
%Recovery ^s		15.8	15.7	115.5	112.0	84.9	47.4

*Ingredients expressed as % of total
^sPercent recovery post-encapsulation.

[0419] As in **Example 5**, *M. elsdenii* (*M. e.*) encapsulated using HPO and the spinning disk method had a recovery of about 16% (Table 12). Cell recovery was greater when the cells did not go through the spinning disk process and only received the overcoat (CW and HCO mixture). Cell recovery, however, decreased with increasing amount of lipid in the overcoat, going down to 47% recovery with the highest lipid level tested of 65%.

Table 13. Formulation composition and recovery post-encapsulation.

	Ingredient*	6	7	8A	9	10	11	12A	13	14	15	16A
Core	FD <i>M. e.</i>	5.2	4.6	4.4	5.2	4.9	4.6	4.4	5.2	4.9	4.6	4.4
	Maltodextrin	26.3	23.4	21.9	26.3	24.8	23.4	21.9	26.3	24.8	23.4	21.9
	HPO	58.5	52.0	48.8	58.5	55.3	52.0	48.8	58.5	55.3	52.0	48.8
	Total Core	90.0	80.0	75.0	90.0	85.0	80.0	75.0	90.0	85.0	80.0	75.0
Coat	CW	-	-	-	3.0	4.5	6.0	7.5	6.0	9.0	12.0	15.0
	HCO	10.0	20.0	25.0	7.0	10.5	14.0	17.5	4.0	6.0	8.0	10.0
	Total Coat	10.0	20.0	25.0	10.0	15.0	20.0	25.0	10.0	15.0	20.0	25.0
	Total Lipid	68.5	72.0	73.8	65.5	65.8	66.0	66.3	62.5	61.3	60.0	58.8
%Recovery [§]		20.0	19.6	22.1	21.2	20.9	22.3	19.2	20.9	22.9	19.2	21.7
*Ingredients expressed as % of total.												
§Percent recovery post encapsulation.												

[0420] Addition of an outer coat using a Wurster, after spinning disk encapsulation with HPO did not significantly affect cell recovery post encapsulation (Table 13), all ranging from 19.2 to 22.9 % compared to 16% for formulations with spinning disk only. Composition of the outer coat, HCO alone or in combination with CW, did not significantly affect cell recovery post-encapsulation.

[0421] Formulations 5, 20, 8, 12, and 16 were then tested in feed using a low pH and high moisture diet (Table 2), to establish in feed survival following procedure described in **Example 1**.

Table 14. *M. elsdenii* recovery after mixing encapsulated product into low pH and high moisture diet for 0 or 4 hours at 25° C or 52° C under aerobic conditions. *Carnauba wax (CW) and Hydrogenated Cottonseed Oil (HCO) were mixed at ratio indicated in parentheses. &Hydrogenated Palm Oil only.

Formulation # & Treatments*		% recovery at 25° C		% recovery at 52° C	
		0 hours	4 hours	0 hours	4 hours
5A	Spinning disk only&	42%	8%	36%	0%
20A	Wurster only (3:7)	4%	0%	-	-
8A	Spinning disk + Wurster (0:1)	49%	39%	56%	18%
12A	Spinning disk + Wurster (3:7)	56%	50%	61%	16%

16A	Spinning disk + Wurster (6:4)	54%	46%	45%	20%
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[0422] Formulations made by using spinning disk only or Wurster only did not provide sufficient protection to *M. elsdenii* during the in-feed mixing and resulted in cell recovery, after 4-hour exposure, lower than 10% regardless of the temperature or method used (Table 14).

[0423] Two-step encapsulation, spinning disk and Wurster, did result in an increase recovery at both 25° C and 52° C compared to either method used individually.

Example 7: Addition of a second overcoat after two-step encapsulation decreased cell recovery post encapsulation.

[0424] It was determined if adding an additional overcoat after the two-step encapsulation would provide additional protection to *M. elsdenii* recovery post-encapsulation.

[0425] *M. elsdenii* culture was grown, cooled, concentrated and freeze-dried following method described in WO 2018/144653 A1. An aliquot of the resulting freeze-dried culture was mixed with Maltodextrin at 1:5 ratio and encapsulated as described in **Example 5** with an additional coating for a total of 33 or 50% of lipid overcoat material. Final formulation compositions are listed in Table 15. Encapsulated products were analyzed for colony forming unit (CFU) and compared to initial freeze-dried powder to determine percent recovery post-encapsulation (Table 15) following protocol described in **Example 1**.

Table 15. Formulations composition and recovery post encapsulation.

Portion	Ingredient*	Particles size (212-710 µm)					
		33/8B	50/8B	33/12B	50/12B	33/16B	50/16B
Core	FD M.e.	3.89	2.90	3.89	2.90	3.89	2.90
	Maltodextrin	19.56	14.60	19.56	14.60	19.56	14.60
	HPO	43.55	32.50	43.55	32.50	43.55	32.50
	Total Core	67.00	50.00	67.00	50.00	67.00	50.00
Coat1	CW	13.40	10.00	6.70	5.00	13.40	10.00
	HCO	8.93	6.67	15.63	11.67	8.93	6.67
Coat2	CW	-	-	3.20	10.00	4.27	13.33
	HCO	10.67	33.33	7.47	23.33	6.40	20.00
	Total Coat	33.0	50.0	33.0	50.0	33.0	50.0
	Total Lipid	76.55	82.50	76.55	82.50	76.55	82.50
	% Recovery ^s	11	13	5	13	14	17

*Ingredients expressed as % of total.
\$Percent recovery post encapsulation.

- [0426] Percent recovery post-encapsulation was low across all formulations. Addition of a second outer layer decreased the cell viability in the process.
- [0427] Formulations 50/8B, 50/12B, and 50/16B were then tested in feed (Table 16) using a low pH and high moisture diet (Table 2) to establish in feed survival following procedure described in **Example 1**.

Table 16. *M. elsdenii* recovery after mixing encapsulated product into low pH and high moisture diet for 0 or 4 hours at 25 or 52° C under aerobic conditions.

Formulation #	% recovery at 25°C		% recovery at 52°C	
	0 hours	4 hours	0 hours	4 hours
50/8B	100%	78%	84%	42%
50/12B	53%	46%	48%	32%
50/16B	69%	52%	56%	37%

- [0428] Formulation 50/8B had the best cell recovery after 4-hour incubation in low pH and high moisture diet at either 25° C or 52° C. Increasing the amount of a second outer coating increased the cell survival when mixed in feed when compared to formulation 8A, 12A, and 16A from **Example 6**.
- [0429] Formulation 33/8B, 33/12B and 33/16B were further tested for shelf life at 4° C for up to 12 months following procedures described in **Example 1**. Results were expressed as CFU/g of formulation. *See* Figure 2. Regardless of the composition of the first or second coat, all formulations with a final 33% lipid coating performed similarly when stored at 4° C for up to 12 months.

Example 8: Optimal lipid content of coating in two-step encapsulation formulations.

- [0430] Next, the optimal level of lipid to be used in the outer coating to provide the highest in feed protection while still allowing for release of the cells in the rumen was determined.
- [0431] *M. elsdenii* culture was grown, cooled, concentrated, and freeze-dried following the method described in WO 2018/144653 A1. An aliquot of the resulting freeze-dried culture was mixed with, or without, maltodextrin (as a carrier) at 1:10 ratio and encapsulated as highlighted in Table 17 and **Example 5**. Formulations were produced to have either a small particle size (213-704 μm) or large particle size (704-1400 μm). Small

particle size was hypothesized to increase surface area and maximize release post-ingestion by the cattle (rumen release).

[0432] Encapsulated products were analyzed for colony forming unit (CFU) and compared to initial freeze-dried powder to determine percent recovery post-encapsulation (Table 17) following the protocol described in **Example 1**.

Table 17. Formulation composition and cell recovery

		Particle Size (213-704 μm)					Particle Size (704-1400 μm)		
	Ingredient*	7A	5A	6A	3A	4A	8A	1A	2A
Core	FD M.e.	3.00	2.25	2.10	2.25	2.10	30.00	22.50	21.00
	Maltodextrin	27.00	20.25	18.90	20.25	18.90	-	-	-
	HPO	70.00	52.50	49.00	52.50	49.00	70.00	52.50	49.00
	Total Core	100.00	75.00	70.00	75.00	70.00	100.00	75.00	70.00
Coat 1	CW	-	-	-	10.00	12.00	-	10.00	12.00
	HCO	-	25.00	30.00	15.00	18.00	-	15.00	18.00
	Total Coat	0	25.00	30.00	25.00	30.00	0	25.00	30.00
	Total Lipid	70.00	77.50	79.00	77.50	79.00	70.00	77.50	79.00
% Recovery[§]		17.31	11.57	12.33	13.55	14.29	20.69	10.27	10.43

*Ingredients expressed as % of total.

[§]Percent recovery post encapsulation.

[0433] The presence of maltodextrin as a carrier in the core did not affect cell recovery post-encapsulation (7A vs. 8A; 3A vs. 1A; 4A vs. 2A). Addition of coating with HCO alone or in combination with CW slightly decreased cell recovery compared to their counterparts made of core only. Formulations resulting in larger particle size had the lowest cell recovery.

[0434] The formulations were then tested for in feed survival at 25° C and 52° C for 4 hours (Table 18), following the protocol described in **Example 1**, and using a low pH and high moisture diet (Table 2) representative of a feedlot diet. Percent recovery after 4 hours incubation in the diet were calculated compared to concentration of encapsulated product prior to mixing in the diet.

Table 18. *M. elsdenii* recovery after mixing encapsulated product into low pH and high moisture diet for 4 hours at 25° C or 52° C under aerobic conditions.

Formulation #	Particle size	Treatments	% recovery after 4 hours at 52°C
1A	213-704 μm	Spinning disk + Wurster (25%; 6:4)*	21.1
2A		Spinning disk + Wurster (30%; 6:4)*	25.8

3A	704-1400 µm	Spinning disk + Wurster (25%; 6:4)*	13.1
4A		Spinning disk + Wurster (30%; 6:4)*	19.7
5A		Spinning disk + Wurster (25%; 1:0)*&	16.9
6A		Spinning disk + Wurster (30%; 1:0)*&	14.5

*Total coat lipid percentage and Hydrogenated Cottonseed Oil/Carnauba wax ratio indicated in between brackets.

&Hydrogenated Cottonseed Oil alone

[0435] All formulations showed full recovery after 4 hours incubation at 25° C in low pH and high moisture diet, regardless of the treatments (data not shown). Formulations containing 25% lipid in the outer coat had slightly lower recovery compared to their counterparts containing 30%. Formulations with smallest particle size displayed a slight improvement in recovery after 4 hours incubation at 52° C in low pH and high moisture diet.

Example 9: Optimal particle size of initial freeze-dried culture.

[0436] Next, standardizing the particle size of the freeze-dried *M. elsdenii* culture prior to two-step encapsulation was analyzed to determine if it would result in greater cell recovery and protection.

[0437] *M. elsdenii* culture was grown, cooled, concentrated and freeze-dried following the method described in WO 2018/144653 A1. Freeze-dried powder was sieved and divided in two groups: < 400 microns and < 1600 microns. Resulting freeze-dried powder were mixed with maltodextrin and encapsulated as highlighted in Table 19 and **Example 5**.

[0438] Encapsulated products were analyzed for colony forming unit (CFU) and compared to initial freeze-dried powder to determine percent recovery post encapsulation as described in **Example 1**.

Table 19. Formulation composition and cell recovery post encapsulation.

	Ingredient*	FD <i>M. elsdenii</i> < 1600 µm			FD <i>M. elsdenii</i> < 400 µm		
		X401	8 (X2)	16(X2)	X402	8(X1)A	16(X1)A
Core	FD M.e.	5.80	4.35	4.35	5.80	4.35	4.35
	Malto	29.20	21.90	21.90	29.20	21.90	21.90
	HPO	65.00	48.75	48.75	65.00	48.75	48.75
	Total Core	100.00	75.00	75.00	100.00	75.00	75.00
Coat 1	CW	---	---	10.00	---	---	10.00
	HCO	---	25.00	15.00	---	25.00	15.00
	Total Coat	0.00	25.00	25.00	0.00	25.00	25.00
Total Lipid		65.00	73.75	73.75	65.00	73.75	73.75

% Recovery[§]	45.25	37.00	38.00	47.41	46.00	36.00
*Ingredients expressed as % of total.						
§Percent recovery post encapsulation						

[0439] Particle size of the freeze-dried *M. elsdenii* culture did not significantly affect cell recovery post-encapsulation. However, consistency in particle size of the initial freeze-dried *M. elsdenii* culture is important to create an efficient encapsulation, as shown by the increased cell recovery observed in this example compared to previous examples. Table 20 compares two step formulations all made of 75% core with maltodextrin and HPO and coated with 25% HCO where the only difference is that the initial freeze dried culture was sieved (8(X1)A and 8(X2) or not (8A and 5A). Recovery in formulations with a homogeneous initial freeze dried culture ranged from 37 to 46%, while the recovery in the same formulations without a pre-screening of the freeze dried culture only had an 11.5 to 22% recovery.

Table 20. Effect of freeze-dried culture pre-screening on final cell recovery post encapsulation.

		FD <i>M. elsdenii</i> < 400 µm	FD <i>M. elsdenii</i> < 1600 µm	No screening	
Portion	Ingredient	8(X1)A*	8 (X2)*	8A[§]	5A^{&}
Core	FD M.e.	4.35	4.35	4.35	4.35
	Malto	21.90	21.90	21.90	21.90
	HPO	48.75	48.75	48.75	48.75
	Total Core	75.00	75.00	75.00	75.00
Coat 1	CW	---	---	---	---
	HCO	25.00	25.00	25.00	25.00
	Total Coat	25.00	25.00	25.00	25.00
Total Lipid		73.75	73.75	73.75	73.75
% Recovery		46.00	37.00	22.10	11.57

*from this example; §from Example 6; &from Example 8.

[0440] Formulation 8 (X) was further tested for in feed survival at 25° C and 52° C for up to 14 hours (Table 21), following protocol described in **Example 1**, and using a low pH and high moisture diet (Table 2). Percent recovery after 4 hours of incubation in the diet were calculated compared to concentration of encapsulated product prior to mixing in the diet.

Table 21. *M. elsdenii* recovery after mixing encapsulated product (8(X1) into low pH and high moisture diet for up to 14 hours at 25° C or 52° C under aerobic conditions.

	Recovery (%) after exposure for various times			
Temperature	4 hours	6 hours	8 hours	14 hours
25° C	92.67%	52.40%	46.90%	32.08%
52° C	22.47%	10.45%	11.92%	7.82%

[0441] After 4 hour incubation in low pH and high moisture diet at 25° C more than 92% of the *M. elsdenii* were still alive and up to 32% remained alive after 14 hours, demonstrating the efficacy of the encapsulation. At 52° C, survival of the cells was lower, but was still far better than core alone (**Example 6**) or other encapsulations previously tested.

Example 10: Passage of formulations through micromachine, prior to in feed exposure, had little impact on *M. elsdenii* recovery

[0442] Next, the impact of passage through the micromachine system on the two-step formulations and their ability to protect the cells when exposed to feed afterward was determined.

[0443] Formulations 8A and 16A from **Example 6** were processed individually through the micromachines (bowl system and continuous flow system) and then mixed in low pH and high moisture diet (Table 2) for 4 hours at room temperature following the protocol described in **Example 1**.

[0444] CFU results were adjusted for amount of product and expressed as CFU/g of product (formulation). Percent recovery after 4 hours incubation in the diet was calculated compared to concentration of encapsulated product prior to passage through micromachine and in feed exposure. Results were compared to the formulation counterpart exposed to the feed only (not processed through the micromachine). Six samples per time point and per treatment.

[0445] Percent recovery (Figure 3) was not affected by the type of micromachine system (Bowl vs. Continuous) or the formulations used (with (16A) or without (8A) carnauba wax in the outer coating). In addition, recovery results with or without passage through the micromachine systems were similar, regardless of the formulations, demonstrating that the passage through the micromachine system had little impact on the formulations ability to protect *M. elsdenii*.

Example 11: Spinning disk HPO encapsulation followed by an outer coating with HCO alone or with CW allowed for delayed, but full cell release after 7-12 hours in *in vitro* fermentation model.

[0446] Next, it was determined if two step encapsulation using spinning disk and Wurster system allowed for full release of the bacteria (*M. elsdenii*) in an *in vitro* fermentation model and if the formulations yield the same level of gas production as an indicator for bacterial growth.

[0447] The ANKOM RF Gas Production System provided an accurate method for monitoring and measuring gas production *in vitro*. Samples of freeze-dried *M. elsdenii* culture, core, formulation 8(X1) (**Example 9**), formulation 16(X1) (**Example 10**) and vegetable oil formulation (**Example 3**) were placed in Pyrex® bottles under sterile conditions. After which 100 mL of semi-defined lactate media was added to each bottle under sterile and anaerobic conditions. The bottles were then fitted with RF pressure sensor modules and placed in a shaker incubator for 24 hours at 39° C. All treatments were prepared in triplicate. The pressure of each bottle was measured at 5-minute intervals and recorded to generate gas production curves in psi (Figure 4). Sample weights were adjusted based on initial sample concentration of *M. elsdenii* to target an equivalent amount of *M. elsdenii* in each bottle.

[0448] Formulations made by two-step encapsulation showed a delayed release (Figure 4) compared to freeze dried culture alone or freeze-dried culture encapsulated in a unique layer of vegetable oil or hydrogenated palm oil (Core). Capacity of the cells to utilize lactate remained unaffected as shown by the similar slopes and the total amount of gas produced for all encapsulated treatments. This experiment further demonstrated the increased protection provided by the two-step formulation over single encapsulation formulations.

Example 12: Spinning disk HPO encapsulation followed by an outer coating with hydrogenated cottonseed oil alone provides sufficient cell protection during exposure to high pH and low moisture feed.

[0449] Next, spinning disk encapsulation was followed by an outer coating with HCO to determine if this process provided enough protection to *M. elsdenii* when mixed with low moisture and high pH diet, and high moisture and low pH diet for up to 4 hours.

[0450] Formulation 8(X1)A from **Example 9** was mixed with a finisher diet with low moisture and high pH (Table 4) and a finisher diet with high moisture and low pH (Table 2) and kept either at 25° C or at 52° C for 4 hours following the protocols described in **Example 1** with the exception that a 15 gram aliquot of diets were used instead of 30 grams. Percent recovery after 4 hours of incubation in the diets (Table 22) was calculated compared to concentration of encapsulated product prior to mixing in the diets.

Table 22. *M. elsdenii* recovery after mixing encapsulated product (8(X1)) into low moisture and high pH and a high moisture and low pH diets for 4 hours at 25° C or 52° C under aerobic conditions.

Diets	% recovery after 4 hours	
	25° C	52° C
Low moisture, high pH diet	74	18
High moisture, low pH diet	100	36

[0451] All *M. elsdenii* cells were recovered (100%) after a 4-hour exposure at room temperature (25° C) to high moisture and low pH diet and only a 26% decay was observed with the low moisture and high pH diet. Recovery after 4 hours in either diet at 52° C was also significantly higher compared to prior formulations tested, showing that formulation 8(X1)A is superior to previously tested formulations.

Example 13: Commercial scale production of *M. elsdenii* encapsulated in a two-step process using palm oil (HPO) and cottonseed oil (HCO).

[0452] The effectiveness of the two-step encapsulation with a hydrogenated palm oil core and a cottonseed oil coat in protecting *M. elsdenii* during storage, delivery, and exposure to a common feedlot diet when produced on a commercial scale was verified.

[0453] Final formulation was prepared by milling the freeze-dried *M. elsdenii* culture obtained following the method described in WO 2018/144653 A1 with a small screen size (Sieve 024R01823) to obtain a consistent powder of < 400 µm. The resulting powder was mixed with maltodextrin and encapsulated with hydrogenated palm oil (HPO) using the spinning disks to form a core. The core was then coated with hydrogenated cottonseed oil (HCO) in Wurster fluid bed agglomerating coater to obtain a final product with particle size ranging from 212 to 710 µm. Final formulation composition is listed in Table 23. A total of three batches were produced on a commercial scale (25 kg) and tested for

recovery post-encapsulation (Table 23), shelf-life at -20° C and 4° C (Figure 5), and infeed survival (Figure 6) following the protocols described in **Example 1**.

Table 23. *M. elsdenii* cell recovery after encapsulation process.

Portion	Ingredient*	8(X1)
Core	FD <i>M. elsdenii</i> < 400 µm	4.35
	Maltodextrin	21.90
	HPO	48.75
	Total Core	75.00
Coat	CW	-
	HCO	25.00
	Total Coat	25.00
	Total Lipid	73.75
CFU/g		6.99 x 10 ¹⁰
% Recovery [§]		55

*Ingredients expressed as % of total.

[§]Percent recovery post encapsulation

- [0454] The final formulation process selected yield on average a 55% recovery for *M. elsdenii* cells post-encapsulation, with 9% variation for the three batches.
- [0455] Average loss of the three batches over the first month of storage at 4° C or -20° C was respectively 0.2 and 0.1 log. Knowing that most losses occur within the first 2 months of storage, data was extrapolated to the 12-months' time point. See Figure 5.
- [0456] Commercial batches were then mixed with two high concentrate diets -- one low pH/high moisture (Table 2) and one high pH/high moisture (Table 3) -- to test in feed survival at 25° C and 52° C for up to 6 hours (Figure 6) as described in **Example 1**.
- [0457] The formulation provided protection to *M. elsdenii* through mixing in feed with a decay of 25% or less for the first 4 hours at 25° C regardless of the diet (Figure 6). In addition, *M. elsdenii* was able to survive in the same diets when exposed to a higher temperature, 52° C, with a recovery of about 30% after a 4-hour exposure (Figure 6). As expected, decay of *M. elsdenii* was greater after 6 hours exposure regardless of diet and temperature. Time spent between feed mixing and distribution to the animal under normal conditions at a commercial feedlot is less than 4 hours, showing that this formulation made of a two-step encapsulation with a hydrogenated palm oil core and a cottonseed oil coat provided sufficient protection for *M. elsdenii* to withstand mixing in feed and feed distribution at a commercial feedlot.

[0458] Commercial batches were further tested for survival through Bowl micromachine system (Table 24) following protocol described in **Example 1**.

Table 24. *M. elsdenii* recovery after passage of formulation batches through the micromachine (Bowl system).

Batches	% Recovery
Batch A	83%
Batch B	91%
Batch C	86%
Average	87%

[0459] On average, 87% of the cells (*M. elsdenii*) survived the passage through the micromachine. As in **Example 10**, passage through the micromachine had little impact on cell recovery.

[0460] In addition, formulation batches were tested to mimic storage in micromachine bins at 25° C, 30° C, and 37° C (Table 25) prior to inclusion in the diet following protocol described in **Example 1**.

Table 25. Recovery following micromachine's bins environmental exposure at 25° C, 30° C, or 37° C.

	25° C				30° C				37° C			
	3h	6h	12h	14h	3h	6h	12h	14h	3h	6h	12h	14h
Batch A	103%	93%	92%	93%	106%	92%	97%	96%	98%	89%	92%	89%
Batch B	74%	78%	79%	82%	77%	84%	84%	78%	75%	78%	77%	81%
Batch C	72%	79%	80%	79%	74%	75%	72%	79%	70%	74%	74%	68%
Average	83%	83%	84%	85%	86%	84%	84%	85%	81%	80%	81%	79%

[0461] Cell loss due to environmental exposure in micromachine's bins appeared to occur within the first 3 hours and were not significantly different regardless of the temperature. Increase in temperature from 25° C to 37° C during the environmental exposure appeared to slightly decrease cell recovery, with an additional 6% cell loss. However, minimal differences were observed when comparing 25° C and 30° C incubation. These data demonstrate that the formulation can withstand storage in the bins for an extended period of time prior to delivery through the micromachine, while still providing 79% or more of live cells.

Example 14: Viability of freeze-dried *M. elsdenii* culture mixed in sterilized ground corn and exposed to environmental conditions (heat and humidity) for up to 4 hours.

[0462] To assess viable *M. elsdenii* potentially ingested by the animals when *M. elsdenii* freeze-dried powder (without any encapsulation) is mixed with ground corn and top dressed on feed.

[0463] In the study presented in WO 2018/144653 A1, freeze-dried *M. elsdenii* powders were top-dressed onto sterilized ground corn in aluminum pans and samples collected after 0, 1, 2, and 4 hours of exposure to ambient atmosphere in the laboratory or outside in the sun. This experiment was repeated 4 times over the course of the months of July and August 2016 (Figure 7) in Wamego, Kansas USA (high humidity and heat).

[0464] Statistical analysis revealed an exposure time by storage conditions interaction ($P = 0.0007$), an exposure time effect ($P < 0.0001$), and a storage conditions effect ($P < 0.0001$). *M. elsdenii* concentration in freeze-dried product mixed with ground corn and exposed to atmosphere in the laboratory numerically decreased during the 4-hour exposure but were not significantly different from their initial concentration. Percent recovery after 4 hours was around 17.4%.

[0465] *M. elsdenii* concentration in the samples exposed to outside conditions decreased much faster than their counterpart kept inside at room temperature and were significantly different after 2 hours exposure. After 4 hours, only 3.8% of the initial *M. elsdenii* cells were still viable on average. *M. elsdenii* concentrations in these samples were very variable from one experiment to the other, as shown by the large standard deviation. The large variations might be attributable to differences in outside conditions (heat and humidity), but also by differences in the freeze-dried product used which may have been more or less resistant to heat and humidity.

[0466] Sterilized ground corn would have a percent humidity far lower than the low pH and high moisture diet or high pH and high moisture diet tested above, and would be considered as a lesser challenge, yet the encapsulated product widely outperformed the results presented here with a percent recovery ranging from 75-78% at room temperature and 28-32% at 52° C after 4 hours exposure in either diet. These results demonstrate the utility and efficiency of the formulation.

Example 15: Evaluation of encapsulated *M. elsdenii* NCIMB 41125 in an accelerated beef step-up program and an acidosis challenge model

[0467] The objective of this study was to evaluate the effects of LactiproNXT® drench (a one-time drench of 1×10¹¹ CFU of *M. elsdenii* NCIMB 41125) or LactiproNXT® drench and daily feeding of encapsulated *M. elsdenii* during an accelerated step-up program, finishing period, and an acidosis challenge on dry matter intake (DMI), *in-vitro* lactate utilization, ruminal lactate and volatile fatty acids (VFA) concentrations.

[0468] **Methods:** Ruminally cannulated British breed crossbred steers (n = 40, initial body weight (BW) 437 ± 98 kg) were individually-fed at the University of Nebraska. Treatments consisted of:

- Control: steers that were fed no *M. elsdenii*
- Drench: steers drenched with the commercial LactiproNXT® at 1×10¹¹ CFU of *M. elsdenii* on day 1 of the experiment and received no other *M. elsdenii*
- Low: steers drenched with a commercial dose of LactiproNXT® on day 1 of the experiment and received encapsulated *M. elsdenii* daily as a top dress to provide an estimated 1×10⁶ CFU to the rumen per head per day.
- Medium: steers drenched with a commercial dose of LactiproNXT® on day 1 of the experiment and received encapsulated *M. elsdenii* daily as a top dress to provide an estimated 1×10⁷ CFU to the rumen per head per day.
- High: steers drenched with a commercial dose of LactiproNXT® on day 1 of the experiment and received encapsulated *M. elsdenii* daily as a top dress to provide an estimated 1×10⁸ CFU to the rumen per head per day.

[0469] Control cattle were adapted to the finisher diet in 18 days while Drench, Low, Medium, and High cattle were adapted in 9 days. Steers were fed once daily at 0700 h using a Calan gate system and had *ad libitum* access to water. The experiment included five continuous phases (Table 26): step-up period (days 1–19); finishing period (days 20–88); feed restriction (day 89, 24-h full feed restriction); challenge period (days 90, cattle were fed at 150% of max DMI from finishing period); and recovery period (days 91–96).

Table 26. Experimental periods with corresponding diets and treatments.

	Period, days				
	Step-up	Finishing	Feed restriction	Challenge	Recovery

Treatment	d1-3	d4-6	d7-9	d10-12	d13-15	d16-18	d19-88	d89	d90	d91-96
Control	Step 1		Step 2		Step 3		Finisher	24-h full feed restriction	fed at 150% of max DMI from finishing period	Back to finisher
Drench	Step 1	Step 2	Step 3	Finisher						
Low	Step 1	Step 2	Step 3	Finisher						
Medium	Step 1	Step 2	Step 3	Finisher						
High	Step 1	Step 2	Step 3	Finisher						

[0470] Feed refusals were collected every 3 days during the step-up period, every 7 days during the finishing period, and every day during challenge and recovery periods. Samples were collected at 0600 h and dried in a forced-air oven to correct for dry matter (DM; Table 27) to determine dry matter intake (DMI).

Table 27. Dietary composition (% DM) from step 1 through the finishing diet.

Ingredient	% of DM			
	Step 1	Step 2	Step 3	Finisher
Steam-flaked corn	37	52	62	70
Modified dried distillers grains plus solubles	18	18	18	18
Alfalfa hay	40	25	15	7
Supplement ¹				
Fine ground corn	2.202	2.202	2.202	2.202
Limestone	1.680	1.680	1.680	1.680
Urea	0.600	0.600	0.600	0.600
Salt	0.300	0.300	0.300	0.300
Tallow	0.125	0.125	0.125	0.125
Beef Trace Mineral Premix ²	0.050	0.050	0.050	0.050
Rumensin-90 Premix ³	0.165	0.165	0.165	0.165
Vitamin A-D-E ⁴	0.015	0.015	0.015	0.015
Tylan-40 Premix ⁵	0.011	0.011	0.011	0.011
Neg, Mcals/d ⁶	61.2	68.5	73.4	77.4
Chemical composition, % ⁷				
Neutral detergent fiber	30.9	24.2	19.7	16.1
Acid detergent fiber	20	14.2	10.4	7.3
Crude protein	16.5	15.2	14.3	13.6
¹ Supplement fed at 5% of dietary DM for all treatments.				
² Premix contained 6.0% Zn, 5.0% Fe, 4.0% Mn, 2% Cu, 0.29% Mg, 0.20% I, and 0.05% Co.				
³ Supplement formulated to provide 33 mg/kg of monensin (Rumensin®, Elanco Animal Health; DM Basis).				
⁴ Premix contained 30,000 IU vitA, 6,000 IU vitD, and 7.5 IU vitE per gram.				
⁵ Supplement formulated to provide 9.8 mg/kg of tylosin (Tylan®, Elanco Animal Health; DM Basis).				
⁶ Based on the 2016 revised NASEM.				
⁷ Based on monthly composites, analyzed for each ingredient (DM basis).				

- [0471] Encapsulated products used in this experiment were prepared as described in **Example 3**, amount of freeze-dried *M. elsdenii* culture and carrier (potato starch) varied to meet the target CFU/head/day (Table 28).

Table 28. Formulation compositions.

Portion	Ingredient*	Target CFU/head/day		
		1x10 ⁶	1x10 ⁷	1x10 ⁸
Matrix	FD <i>M. elsdenii</i>	0.189	1.89	18.90
	Carrier (Potato Starch)	39.811	38.11	21.10
	Total Matrix	40.00	40.00	40.00
Coat	Vegetable oil@	60.00	60.00	60.00
	Total Coat/Lipid	60.00	60.00	60.00

*Ingredients expressed as % of total.
 @mixture of mono- and di-glycerides of palmitic, stearic, oleic, linoleic, and linolenic acids.

- [0472] Rumen fluid samples were collected every 3 days in the step-up period, every 7 days in the finishing period, and every day during challenge and recovery periods at 1300 h. During the challenge and recovery periods (days 88, 90, 91, and 92), a small tube of rumen fluid collected was retained at room temperature and 0.1 mL of the fluid was inoculated into hungate tubes containing a semi-defined lactate media to estimate lactate disappearance. A total of three tubes per day per animal were inoculated at 1400 h. Tubes were incubated in a 38° C water bath for either 0, 12, and 24 hours for day 88 and for days 90–92 at 0, 12, and 18 hours, then frozen for analysis of lactate and VFA using gas chromatography.
- [0473] Repeated measures were used within three phases of step-up period (days 1–19), finishing period (days 20–88), and recovery period (days 91–93). Data were tested for linear and quadratic effects of dose with drench as the intercept. Data were tested for linear and quadratic effects of time tested and time × treatment interaction tested using covariate regression. The following contrast were reported Control vs Lactipro (cattle fed any *M. elsdenii*) and Drench vs daily (Low, Medium, and High). Proc IML was used to get contrast coefficient for unequal spacing. Statistical significance was declared at $P \leq 0.10$ and a tendency $P \leq 0.15$.
- [0474] **Results:** No differences were observed for DMI in the step-up, finishing, or challenge period (Table 29). During the recovery period, DMI increased by 4.6% for Low, Medium, and High compared to Drench ($P = 0.07$) and DMI expressed as a

percentage of pre-challenge intake increased by an average of 17.5% ($P = 0.05$) for Low, Medium, and High compared to Drench.

Table 29. Dry matter intake of ruminally cannulated steers dosed with *M. elsdenii*.

		Treatments						P-value			
		Control	Drench	Low	Medium	High	SEM	Control vs. M. e.	Drench vs. Daily	Linear	Quadratic
DMI [#]	Step-up [@]	6.9	7	7.4	6.8	7	0.28	0.12	0.32	0.42	0.42
DMI, kg	Step-up [@]	9.6	9.4	9.5	9.4	9.2	0.26	0.51	0.84	0.71	0.45
	Finishing [@]	13.1	12.1	12.7	12.8	12.5	0.54	0.36	0.48	0.69	0.16
	Challenge [@]	20.6	19.8	21.5	20.9	21.8	1.35	0.76	0.3	0.3	0.99
	Recovery [@]	10.4	8.8	12	10.8	10.9	1.12	0.85	0.07	0.11	0.26
DMI, %*	Recovery	78.3	68.8	88.7	83.2	86.9	7.7	0.64	0.05	0.06	0.57

[#]Neg Mcals/d

[@]Step-up d 1-18; Finishing d 20-88; Challenge d 90; Recovery d 91, 92, & 93

*Percent of pre-challenge intake, expressed as % of average intake of the 9 d immediately before challenge.

[§]Linear effect of dose (Low, Medium, & High) with Drench as the intercept.

[&]Quadratic effect of dose (Low, Medium, & High) with Drench as the intercept.

[0475] *In vitro* lactate utilization on day 88 (pre-challenge) was significantly increased in the daily dosed treatments, regardless of the dose (Low: 98.3, Medium: 82.2, and High: 87.8 mmol/L) after 12 hour incubation compared to the Drench (130.8 mmol/L) and Control treatments (126.8 mmol/L; $P \leq 0.05$, Table 30).

Table 30. Lactate utilization pre-challenge (day 88).

		Lactate concentration, mmol/L					P-value		
Incubation time, h		Control	Drench	Low	Medium	High	SEM	Treatment	Hour
0		146.6	148.2	145.0	143.7	145.3	10.66	0.13	<0.01
12		126.8 ^a	130.8 ^a	98.3 ^b	82.2 ^b	87.8 ^b	10.66		
24		0.0	0.0	0.0	0.0	0.0	10.66		

^{a,b}Means within a row that lack a common superscript are different ($P \leq 0.05$).

[0476] On days 90, 91, and 92 (Table 31) at 12 hours, a treatment effect was observed with steers in the Low (83.8 mmol/L), Medium (54.0 mmol/L), and High (78.7 mmol/L) treatments having greater lactate utilization than Control (102.6 mmol/L; $P \leq 0.05$).

Table 31. Lactate utilization post-challenge (day 90, 91, and 92).

		Lactate concentration, mmol/L					P-value		
Incubation time, h		Control	Drench	Low	Medium	High	SEM	Treatment	Hour
0		146.8	145.9	146.2	144.8	144.8	8.64	0.14	<0.01
12		102.6 ^a	97.0 ^{a,b}	83.8 ^b	54.0 ^c	78.7 ^b	8.64		

18	10.4	23.2	29.1	13.7	9.2	8.64			
^{a,b,c} Means within a row that lack a common superscript are different ($P \leq 0.05$).									

[0477] During the step-up period, cattle given *M. elsdenii* had 3% greater butyrate ($P < 0.01$) and 4% less total VFA ($P = 0.06$) compared to Control (Table 32). During the feeding period, there tended to be a 3% increase in butyrate for *M. elsdenii* cattle compared to Control ($P = 0.15$). During the recovery period, cattle fed *M. elsdenii* daily had a 10% increase in total VFA concentration compared to the Drench steers ($P = 0.08$).

Table 32. Butyrate and total VFA concentrations from ruminally cannulated steers dosed with *M. elsdenii* during an accelerated step-up and acidosis challenge.

	Treatments					SEM	P-value			
	Control	Drench	Low	Medium	High		Control vs. M. e.	Drench vs. Daily	Linear ^s	Quadratic ^{&}
Butyrate, mM										
Step-up	9.4 ^a	9.3 ^a	8.7 ^a	10.3 ^b	10.2 ^b	0.553	<0.01	0.31	0.41	0.43
Finishing	8.8	9.7	9.3	9.1	9.6	0.446	0.15	0.49	0.57	0.51
Challenge	5.3	6.8	4.9	6.7	7	0.927	0.27	0.61	0.89	0.11
Recovery	8.4 ^a	8.2 ^a	8.0 ^a	10.2 ^b	9.1 ^a	0.978	0.65	0.45	0.36	0.6
Total VFA, mM										
Step-up	112.8 ^b	113.2 ^b	105.0 ^a	104.9 ^a	109.7 ^{a,b}	2.377	0.06	0.01	0.04	0.04
Finishing	130.1	125.8	131.4	135.4	132.3	4.835	0.39	0.93	0.65	0.1
Challenge	135.1	135.9	142.8	140.2	141.4	7.353	0.55	0.54	0.6	0.81
Recovery	118.8 ^a	118.7 ^a	134.1 ^b	134.1 ^b	126.5 ^{a,b}	6.247	0.16	0.08	0.13	0.2
^{a,b} Means within a row that lack a common superscript are different ($P \leq 0.10$).										
^s Linear effect of dose (Low, Medium, & High) with Drench as the intercept.										
^{&} Quadratic effect of dose (Low, Medium, & High) with Drench as the intercept.										

[0478] Conclusions: An accelerated step-up (9 days vs. 18 days for Control) was possible with both the Drench and daily feeding of *M. elsdenii*, regardless of the concentration of cells used. Results suggest however that the one-time LactiproNXT[®] drench (Drench) does not last up to 90 days in the rumen, and thus, cattle experiencing an acidosis challenge late in the feeding period may benefit from the *M. elsdenii* top dress fed daily in addition to the drench. Steers fed *M. elsdenii* daily, tended to have a greater DMI after the acidosis event occurred. They also consumed more feed sooner after an off-feed event, regardless of the *M. elsdenii* concentration fed (Low, Medium, or High), probably due to a greater lactate utilization. Feeding *M. elsdenii* daily could be beneficial

for commercial feedlot and dairy during a winter storm, reimplant day, or any event that causes changes or delays in the cattle's feeding schedule.

- [0479] Formulations used in this study were made of a single layer encapsulation (**Example 3**) which was shown to be outperformed by the two-step encapsulation using HPO and HCO, when looking at recovery post-encapsulation, in feed survival, and rumen release. Therefore, it can only be expected that the two-step encapsulation formulation to result in similar or greater results when used in an animal trial.

Example 16: Using *M. elsdenii* upfront drench results in improved operational efficiency in commercial feedyards.

- [0480] The primary objective of this example was to quantify the operational efficiencies by using LactiproNXT® (*M. elsdenii*) to shorten the step-up period in a commercial feedyard.
- [0481] Cattle feeders generally try to transition cattle to a finishing ration as soon as possible without causing acidosis or bloat. The traditional step-up period takes 21 days, or more, to give time to the normal population of lactic acid-utilizing bacteria to grow in the rumen and to handle the lactic acid produced from starch degradation by lactic acid producing bacteria. During that step-up period, the feedyard will slowly increase the level of starch and decrease the level of roughage using a succession of diets (as illustrated in **Example 15** for the control group), resulting in an increase of the number of loads (diets) to be manufactured, of the number of delivery trips to be performed by the feeding trucks, and of the amount of roughage to be grinded and stored at the facility.
- [0482] LactiproNXT® delivers an immediate, viable population of *M. elsdenii* directly into the rumen at the time of administration, therefore "bypassing" the rumen adaptation period. LactiproNXT® acts as a management tool that allows feedyard managers to start cattle on the finishing ration (low in roughage and high in starch) sooner, allowing yards to improve operational efficiencies. Feedyard managers, using LactiproNXT® to shorten the step-up period, have reported: (1) fewer loads of step-up diets manufactured, (2) fewer trips through the yard delivering starter diets, (3) less roughage required, and (4) less hay to grind, while maintaining or exceeding animal performance.
- [0483] Two commercial feedyard studies (Table 33) were conducted using a similar protocol to evaluate the impact of a shortened step-up period on operational efficiency. An independent firm assessed changes in each yard as a result of shortening the step-up

period by 10 days with the addition of LactiproNXT[®] as an up-front drench (one time application). Monitoring and evaluations were conducted over a 3–4 day period onsite at each feedyard to assess all aspects of cattle feeding, and feed manufacturing and delivery. Operational engineers from the firm gathered insights from feedyard staff about their practices, collected primary data, and created a proprietary model to analyze the results. The case studies presented are actual results and are based on a conservative model estimate. Live and carcass performance was also measured as part of these studies and is reported separately. Actual results may vary from the predicted model. This model continues to be improved in robustness, accuracy, and precision.

Table 33. Feedyard description, assumptions and operational efficiencies for case study 1 and 2.

	Case study 1	Case study 2
Feedyard description		
One time capacity	55,000 head	22,000 head
Normal step-up	21 days	20 days
Feeding protocol	Two ration blend	Two ration blend
Assumptions		
Step-up with LactiproNXT	10 days	10 days
LactiproNXT use	100% of yard	100% of yard
Days on feed; # of turns of cattle/year	180 days on feed; two turns of cattle	180 days on feed; two turns of cattle
Starter vs. finisher hay %	31% vs. 7%	24% vs. 0%
Hay shrink	4%	4%
Operational efficiencies		
Miles not driven	1,615 miles	826 miles
Loads saved*	1,643 loads	876 loads
Metric tons CO ₂ ^s saved	52 metric tons	25 metric tons
Mill hours saved	1,320 hours	628 hours
Tons of hay saved	3,249 T	1,141 T
Tons of hay shrinkage saved	100 T	45 T
Recaptured value^s	\$117,872	\$88,835
*Feed loads saved is a net number and accounts for loads of finishing ration to replace the starter ratio. ^s including cost of LactiproNXT.		

[0484] In conclusion, using LactiproNXT[®] to shorten the step-up period can help feedyards significantly improve operational efficiencies (Table 33). The feedyards analyzed in the case studies used less hay, resulting in less maintenance and labor, fewer trips through the feedyard, decreased utility use and fewer hours spent feeding—providing opportunities to reallocate labor to other feedyard tasks. The net savings includes the annual cost of LactiproNXT[®] and the cost of finishing diet to replace the

starter diet. The use of LactiproNXT[®] provided operational efficiency improvements and a net positive dollar return for the feedyards.

[0485] As shown in **Example 15**, daily administration of encapsulated *M. elsdenii* in feed proved to have additional benefit to the animals and would also include additional operational savings, as it would limit the number of ration changes during a winter storm, reimplant day, or any event that causes variations or delays in the cattle's feeding schedule.

Example 17: Accelerated finishing diet programs that include an on-arrival drench with LactiproNXT[®] and either 1×10^6 or 1×10^7 CFU of *M. elsdenii* in the feed daily in steers showed improvement in cattle performance over a conventional finishing program without LactiproNXT[®].

[0486] As mentioned in **Example 16**, roughage acquisition, handling, feeding, and the need for multiple diets to be milled at the feed yard are common challenges associated with traditional step-up program at commercial feed yards. Additionally, **Example 15** demonstrates that cattle given *M. elsdenii* daily as a top-dress on the feed had rumen fluid that was more efficient at metabolizing lactate *in vitro*, and that the cattle given the bacteria daily were able to regain their pre-challenge intake faster relative to the control and Lactipro drench only treatment. Therefore, accelerating the step-up period with *M. elsdenii* followed by daily inclusion of *M. elsdenii* in feed could improve operational efficiency as well as animal performance.

[0487] The objective of this study will be to compare performance of cattle subjected to a conventional feeding program with cattle in a program that includes an accelerated finishing diet adaption program with a LactiproNXT[®] drench on arrival and daily administration of encapsulated *M. elsdenii* through the feed at either 1×10^6 or 1×10^7 CFU/head/day.

[0488] Approximately 1,600 - 2,000 steers of 12 to 24 months of age and 650 - 850 lbs. will be used in this experiment. Cattle will be housed in dry lot (dirt floor) pens with no enclosure. Cattle will receive one of the following three treatments:

[0489] Control – Traditional step-up program with no *M. elsdenii*.

[0490] *M. e.* Low – *M. elsdenii* (rehydrated drench; 1×10^{10} CFU of *M. elsdenii* NCIMB 41125; LactiproNXT[®]) will be administered at initial processing, cattle stepped-up in

approximately 50% of the days of traditional step-up protocol, and encapsulated *M. elsdenii* will be administered daily to deliver an estimated 1×10^6 CFU per head daily.

[0491] *M. e. High – M. elsdenii* (rehydrated drench; 1×10^{10} CFU of *M. elsdenii* NCIMB 41125; LactiproNXT®) will be administered at initial processing, cattle stepped-up in approximately 50% of the days of traditional step-up protocol, and encapsulated *M. elsdenii* will be administered daily to deliver an estimated 1×10^7 CFU per head daily.

[0492] Encapsulated *M. elsdenii*: *M. elsdenii* culture was grown, cooled, concentrated, and freeze-dried following method described in WO 2018/144653 A1. Freeze dried powder was sieved to < 400 microns and mixed with maltodextrin as shown in Table 34 before being encapsulated to form a core containing the freeze-dried (FD) *M. elsdenii*, maltodextrin, and hydrogenated palm oil (HPO) using spinning disk method. The core was processed to create small particles and then spray coated in a Wurster fluid bed agglomerating coater with hydrogenated cottonseed oil (HCO). Final formulation compositions are listed in Table 34. Final encapsulated cells were further mixed with a carrier (maltodextrin) to meet the target delivery of 1×10^6 or 1×10^7 CFU/50 mg/head/day.

Table 34. Formulation composition and cell recovery post encapsulation.

	Ingredient	Formulation
Core	FD M.e.	4.35
	Malto	21.90
	HPO	48.75
	Total Core	75.00
Coat 1	CW	---
	HCO	25.00
	Total Coat	25.00
Total Lipid		73.75

[0493] Encapsulated products will be stored at -20° C and distributed daily using a continuous flow micromachine.

[0494] All diets will be formulated to meet or exceed the nutritional requirements of the animals. The dry matter (DM) ingredient composition and calculated and/or measured nutrient composition of the diet(s) will be reported. Dry matter and nutrient analysis (including starch content) of the ration will be conducted. Daily records of weight of feed delivered and head counts will be kept on a pen basis for the duration of the trial. Feed refusal will be removed, weighed, and recorded. All cattle will have access to water to

ensure *ad libitum* intake. Cattle will be fed via a 4-step up diet adaptation as indicated in Table 35.

Table 35. Days on feed with corresponding diets for each treatment.

	Days on feed, d										
	d1-2	d3	d4-6	d7	d8-10	d11	d12-13	d14	d15-20	d21	d22-25
Control	Step1	Step1	Step1	1/2*	Step2	Step2	Step2	2/3*	Step3	3/4*	Step4
<i>M. e</i> Low	Step1	1/2*	Step2	2/3*	Step3	3/4*	Step4	Step4	Step4	Step4	Step4
<i>M. e</i> High	Step1	1/2*	Step2	2/3*	Step3	3/4*	Step4	Step4	Step4	Step4	Step4
*Step ration mixed 50/50											

[0495] Cattle will be maintained in groups based on date of arrival, source, and/or truckload until randomization. As soon as enough cattle have been received to fill a full replicate (3 pens), cattle will be placed randomly into pens and one pen will be assigned to each treatment. The process will be repeated 8 times to have 8 pen per treatment. The experimental design will be a Randomized Complete Block Design. The blocking factor will be the three-pen replication, which represents one pen from each treatment per replicate (i.e. 8 blocks). Pen will serve as the experimental unit and block will serve as a fixed or random effect depending on the nature of the variable.

[0496] Following randomization and prior to initial processing, all pens within a replication will be weighed across a platform scale and that weight will serve as the starting study weight. A separate weight for each pen will also be collected at 90 to 95 days on feed. Following the collection of that weight, cattle will be held off feed and water for 4 hours prior to being placed back in their home pen. This is meant to simulate a reimplant or terminal processing event.

[0497] The duration of the trial will be approximately 180 days. On day 180 of the trial, all pens of cattle will be weighed across a pen scale and that weight, minus a 4% shrink factor, will serve as the final weight for the trial. Carcass data will be collected by a third party data collection service.

[0498] Lactic acid utilization assay - Lactic acid metabolism activity of rumen fluid will be measured in cattle after 13 and 14 days on feed. Briefly, cattle will be selected at random in the "Control" and "*M.e.* High" treatment pen (10 animals/treatment; 1-2 animals/pen), brought up to the processing facility and a rumen fluid sample will be taken via an esophageal tube using an electronic peristaltic pump. Sample will be strained through 4 layers of cheesecloth, transferred to pre-labeled 500 mL conical tubes and the lid will be closed tightly. Twelve 0.1 mL of the rumen fluid sample will be inoculated

into 12 Hungate tubes containing a semi-defined lactate media. Tubes will be incubated in a water bath kept at 40° C. After 0, 3, 6, 9, 12, and 15 hours of incubation, two tubes will be removed, optical density measured, and tubes will be placed in a -20° C freezer.

Frozen tubes will be sent to a third-party lab for assessment of lactic acid concentration.

[0499] In addition to the lactic acid utilization, the following response variables will be measured and analyzed: pulls (number and type), mortality, initial weight, final weight, average daily gain, total weight gain, dry matter intake, feed efficiency and carcass characteristic.

[0500] Treatment differences will be determined by running contrast statements comparing the control treatment to the average of the *M.e.* Low and *M.e.* High treatments, and a contrast statement comparing the *M.e.* Low and *M.e.* High treatments.

[0501] **Results:** Cattle given *M. elsdenii* will have greater butyrate production compared to Control regardless of the feeding period. They will also have greater DMI following the reimplant or terminal processing event compared to control. No differences will be observed for DMI in the step-up and finishing period. The *in vitro* lactate utilization will be significantly increased in *M. elsdenii* treatments compared to the control, regardless of the dose.

[0502] **Conclusions:** An accelerated step-up is possible with both daily feeding of *M. elsdenii*, regardless of the concentration used. Steers fed *M. elsdenii* daily consumed more feed sooner after an off-feed event, regardless of the *M. elsdenii* concentration fed (Low or High), probably due to a greater lactate utilization compared to Control. In addition, *M. elsdenii* Low and High treatments had superior or equal cattle performance compared to control. Feeding *M. elsdenii* daily is beneficial for commercial feedlot and dairy during a winter storm, reimplant day, or any event that causes changes or delays in the cattle's feeding schedule.

Example 18: Production of anaerobic cells encapsulated in a two-step process using palm oil (HPO) and cottonseed oil (HCO).

[0503] Anaerobic bacteria can be divided into three categories, (1) obligate anaerobes; (2) aerotolerant anaerobes; and (3) facultative anaerobes. Obligate anaerobes are bacteria that do not survive in normal atmospheric concentrations of oxygen. Some obligate anaerobes can survive in up to 8% oxygen, while others cannot survive unless the oxygen concentration is less than 0.5%. Aerotolerant anaerobes can survive in the presence of

oxygen, but do not utilize oxygen for growth. Facultative anaerobes are able to use oxygen for aerobic respiration but can also use anaerobic respiration if no oxygen is present.

[0504] Megasphaera, such as *M. elsdenii*, Fibrobacter, such as *F. succinogenes*, Butyrivibrio, such as *B. fibrisolvens*, Ruminococcus, such as *R. flavefaciens*, and Bifidobacterium, such as *B. breve* are representative species of obligate anaerobes. Lactobacillus, such as *L. plantarum* and Bifidobacterium, such as *B. animalis* subsp. *lactis* are representative species of aerotolerant anaerobes. Pediococcus, such as *P. acidilactici* and Lactobacillus, such as *L. casei* are representative species of facultative anaerobes.

[0505] This example demonstrates the applicability of the above formulations for other anaerobic bacteria listed above. It also demonstrates the effectiveness of the two-step encapsulation with a hydrogenated palm oil core and a cottonseed oil coat in protecting anaerobic bacteria during storage, delivery, and exposure to a common feedlot diet.

[0506] Formulation was prepared by milling the dried anaerobic bacteria culture with a small screen size (Sieve 024R01823) to obtain a consistent powder of < 400 µm. The resulting powder was mixed with a carrier, such as maltodextrin, and encapsulated with hydrogenated palm oil (HPO) using the spinning disks to form a core. The core was then coated with hydrogenated cottonseed oil (HCO) in a Wurster fluid bed agglomerating coater to obtain a final product with particle size ranging from 212 to 710 µm. Final formulation composition is listed in Table 36. Formulations were tested for recovery post-encapsulation (Table 36), shelf-life, in feed survival, and rumen release following protocols described in **Example 1**.

Table 36. Anaerobic cell recovery after encapsulation process.

Portion	Ingredient*	8(X1)
Core	FD anaerobic cells < 400 µm	4.35
	Carrier (i.e. Maltodextrin)	21.90
	HPO	48.75
	Total Core	75.00
Coat	CW	-
	HCO	25.00
	Total Coat	25.00
	Total Lipid	73.75
% Recovery^S		>10
*Ingredients expressed as % of total.		
^S Percent recovery post encapsulation		

- [0507] Final formulation yield on average at least a 10% recovery for anaerobic bacteria cell post encapsulation.
- [0508] Formulations will be packaged, stored at -20° C and sampled at pre-determined time point to assess shelf-life following the protocol described in **Example 1**.
- [0509] Like for *M. elsdenii* in **Example 13**, average loss over the first month of storage at -20° C will be less than 0.3 log. Knowing that most losses occur within the first few months of storage, loss after 12-month of storage at -20° C will be less than 0.5 log. Two-step encapsulation will provide stable shelf life, regardless of the type of anaerobic bacteria tested.
- [0510] Formulations were then mixed with two high concentrate diets -- one low pH/high moisture diet (Table 2) and one high pH/high moisture diet (Table 3) -- to test in feed survival at 25° C and 52° C for up to 6 hours as described in **Example 1**.
- [0511] The formulation will provide protection to the anaerobic cells through mixing in feed with a decay of 25% or less for the first 4 hours at 25° C, regardless of the diet. In addition, anaerobic cells will be able to survive in the same diets when exposed to a higher temperature, 52° C, with a recovery of 30% or more after a 4-hour exposure. It will be expected that decay of anaerobic cells will be greater after 6-hour exposure regardless of diet and temperature. Time spent between feed mixing and distribution to the animal under normal conditions at a commercial feedlot is estimated to be less than 4 hours, showing that the formulation will provide sufficient protection for anaerobic cells to withstand mixing in feed and feed distribution at a commercial feedlot.
- [0512] Formulations will then tested for rumen release following protocol described in **Example 1** to confirm that anaerobic cells are consistently released in the rumen after ingestion.
- [0513] Rumen release measured for the formulation(s) will fall within the commercial criteria: 50% to be released within the 24-hour *in situ* incubation in rumen of steers fed high concentrate or high forage diets respectively.

Formulations will further be tested for survival through micromachine and environmental exposure in the micromachine's bins following protocol described in Example 1.

[0514] On average, at least 70% of the anaerobic cells will survive the passage through the micromachine and at least 80% of the cells were recovered when left in the bins at 25° C, 30° C, or 37° C for up to 14 hours.

Example 19 – Production of anaerobic encapsulated cells in a two-step process using freeze dried or spray dried cells.

[0515] This example demonstrates the applicability and effectiveness of a two-step encapsulation with an hydrogenated palm oil core and a cottonseed oil coat for *M. elsdenii* and other anaerobic bacteria (see **Example 18**) produced by freeze drying (PCT Pub. No. WO 2018/144653 A1) or spray drying (PCT App. No. PCT/US2023/028982).

[0516] *M. elsdenii* and other anaerobic bacteria culture were grown, cooled, concentrated and either freeze-dried following method described in WO 2018/144653 A1 or spray dried following method described in PCT App. No. PCT/US2023/028982. Resulting dried cultures were milled with a screen to obtain homogeneous powders. The resulting powders were mixed with a carrier, such as maltodextrin, and encapsulated with hydrogenated palm oil (HPO) using spinning disks to form a core. The core was then coated with hydrogenated cottonseed oil (HCO) to obtain a final product with particle size ranging from 212 to 710 µm. Final formulation composition is listed in Table 37. Formulations were produced and tested for recovery post encapsulation (Table 37), shelf-life, infeed survival, and rumen release following protocols described in **Example 1**.

Table 37. Anaerobic cell recovery after encapsulation process.

Portion	Ingredient*	Freeze dried cells	Spray dried cells
Core	Dried anaerobic cells	4.35	4.35
	Carrier (i.e. Maltodextrin)	21.90	21.90
	HPO	48.75	48.75
	Total Core	75.00	75.00
Coat	CW	-	-
	HCO	25.00	25.00
	Total Coat	25.00	25.00
	Total Lipid	73.75	73.75
% Recovery^s		>10	>10

*Ingredients expressed as % of total.

§Percent recovery post encapsulation

- [0517] Regardless of the method used to produce the dried culture, final formulation will yield on average at least a 10% recovery for all anaerobic bacteria cell tested post encapsulation.
- [0518] Formulations will be packaged, stored at -20° C and sampled at pre-determined time point to assess shelf-life following protocol described in **Example 1**.
- [0519] Like for *M. elsdenii* in **Example 13**, average loss over the first month of storage at -20° C will be less than 0.3 log. Knowing that most losses occur within the first few months of storage, loss after 12-month of storage at -20° C will be less than 0.5 log. Method used to produce the initial dried culture will have no effect on shelf life, regardless of the temperature.
- [0520] Formulations will then mixed with two high concentrate diets -- one low pH/high moisture diet (Table 2) and one high pH/high moisture diet (Table 3) -- to test in feed survival at 25° C and 52° C for up to 6 hours as described in **Example 1**.
- [0521] Formulation will provide protection to the anaerobic cells through mixing in feed with a decay of 25% or less for the first 4 hours at 25° C, regardless of the diet or the method used to dry the initial culture. In addition, anaerobic cells will be able to survive in the same diets when exposed to a higher temperature, 52° C, with a recovery of 30% or more after a 4-hour exposure. As expected, decay of anaerobic cells will be greater after 6-hour exposure regardless of diet and temperature. Time spent between feed mixing and distribution to the animal under normal conditions at a commercial feedlot is estimated to be less than 4 hours, showing that the formulation will provide sufficient protection for anaerobic cells to withstand mixing in feed and feed distribution at a commercial feedlot, regardless of the method used to dry the initial culture.
- [0522] Formulations will then be tested for rumen release following protocol described in **Example 1** to confirm that anaerobic cells are consistently released in the rumen after ingestion.
- [0523] Rumen release measured for the formulation(s) will fall within commercial criteria: 50% to be released within the 24-hour *in situ* incubation in rumen of steers fed high concentrate or high forage diets respectively and regardless of the method used to dry the initial culture.

[0524] Formulations will further be tested for survival through micromachine and environmental exposure in the micromachine's bins following protocol described in **Example 1**.

[0525] On average, at least 70% of the anaerobic cells will survive the passage through the micromachine and at least 80% of the cells were recovered when left in the bins at 25, 30, or 37° C for to 14 hours again regardless of the method used to dry the initial culture.

Example 20: Carrier used in the core had little impact on efficiency of the two-step encapsulation, using palm oil (HPO) and cottonseed oil (HCO), of anaerobic cells.

[0526] This Example will demonstrate that using maltodextrin, sucrose, or starch had little to no effect on the effectiveness of the two-step encapsulation with a hydrogenated palm oil core and a cottonseed oil coat in protecting anaerobic bacteria during storage, delivery, and exposure to a common feedlot diet.

[0527] *M. elsdenii* and other anaerobic bacteria culture (See **Example 18**) were grown, cooled, concentrated, and freeze-dried following method described in WO 2018/144653 A1. Resulting dried cultures were milled with a screen to obtain homogeneous powders. The resulting powders were mixed with a carrier, such as maltodextrin, sucrose, or starch, and encapsulated with hydrogenated palm oil (HPO) using the spinning disks to form a core. The core was then coated with hydrogenated cottonseed oil (HCO) to obtain a final product with particle size ranging from 212 to 710 µm. Final formulation compositions are listed in Table 38. Formulations were tested for recovery post-encapsulation (Table 38), shelf-life, in feed survival, and rumen release following protocols described in **Example 1**.

Table 38. Anaerobic cell recovery after encapsulation process.

Portion	Ingredient*	Maltodextrin	Sucrose	Starch
Core	FD anaerobic cells < 400 µm	4.35	4.35	4.35
	Carrier	21.90	21.90	21.90
	HPO	48.75	48.75	48.75
	Total Core	75.00	75.00	75.00
Coat	CW	-	-	-
	HCO	25.00	25.00	25.00
	Total Coat	25.00	25.00	25.00
	Total Lipid	73.75	73.75	73.75
	% Recovery[§]	>10	>10	>10
*Ingredients expressed as % of total.				
§Percent recovery post encapsulation				

- [0528] Final formulations for all will yield on average at least a 10% recovery for anaerobic bacteria cell post encapsulation, regardless of the carrier used in the core.
- [0529] Formulations will be packaged, stored at -20° C, and sampled at pre-determined time point to assess shelf-life following protocol described in **Example 1**.
- [0530] Like for *M. elsdenii* in **Example 13**, average loss of the three batches over the first month of storage at -20° C will be less than 0.3 log. Knowing that most losses occur within the first few months of storage, loss after 12-month of storage at -20° C will be less than 0.5 log. Carrier will not have a significant effect on shelf life regardless of the temperature or bacteria used.
- [0531] Formulations were then mixed with two high concentrate diets -- one low pH/high moisture diet (Table 2) and one high pH/high moisture diet (Table 3) -- to test in feed survival at 25° C and 52° C for up to 6 hours as described in **Example 1**.
- [0532] The formulation will provide protection to the anaerobic cells through mixing in feed with a decay of 25% or less for the first 4 hours at 25° C, regardless of the diet. In addition, anaerobic cells will be able to survive in the same diets when exposed to a higher temperature, 52° C, with a recovery of 30% or more after a 4-hour exposure. As expected, decay of anaerobic cells will be greater after 6-hour exposure regardless of diet and temperature. Time spent between feed mixing and distribution to the animal under normal conditions at a commercial feedlot is estimated to be less than 4 hours, showing that the formulation will provide sufficient protection for anaerobic cells to withstand mixing in feed and feed distribution at a commercial feedlot regardless of the carrier used in the core.
- [0533] Formulations will then be tested for rumen release following protocol described in **Example 1** to confirm that anaerobic cells are consistently released in the rumen after ingestion.
- [0534] Rumen release measured for the formulation(s) will fall within commercial criteria: 50% to be released within the 24-hour *in situ* incubation in rumen of steers fed high concentrate or high forage diets respectively regardless of the carrier used in the core.
- [0535] Formulations will further be tested for survival through micromachine and environmental exposure in the micromachine's bins following protocol described in **Example 1**.

[0536] On average, at least 70% of the anaerobic cells will survive the passage through the micromachine and at least 80% of the cells were recovered when left in the bins at 25° C, 30° C, or 37° C for to 14 hours, regardless of the carrier used in the core.

Example 21: All lipids tested to form the core provided sufficient protection for the two-step encapsulation of anaerobic cells to be commercially viable.

[0537] This Example demonstrates that using palm oil, vegetable oil, stearic acid or Dritex to make the core did not affect the effectiveness of the two-step encapsulation with a cottonseed oil coat in protecting anaerobic bacteria during storage, delivery, and exposure to common feedlot diet.

[0538] *M. elsdenii* and other anaerobic bacteria cells (See **Example 18**) were grown, cooled, concentrated, and freeze-dried following method described in WO 2018/144653 A1. Resulting dried cultures were milled with a screen to obtain homogeneous powders. The resulting powders were mixed with a carrier, such as maltodextrin, and encapsulated with hydrogenated palm oil (HPO), vegetable oil, stearic acid, or Dritex using the spinning disks to form a core. The core was then coated with hydrogenated cottonseed oil (HCO) to obtain a final product with particle size ranging from 212 to 710 μm . Final formulation composition is listed in Table 39. Formulations were tested for recovery post encapsulation (Table 39), shelf-life, in feed survival, and rumen release following protocols described in **Example 1**.

Table 39. Anaerobic cell recovery after encapsulation process.

Portion	Ingredient*	HPO	Vegetable oil	Stearic acid	Dritex
Core	FD anaerobic cells < 400 μm	4.35	4.35	4.35	4.35
	Maltodextrin	21.90	21.90	21.90	21.90
	Lipid	48.75	48.75	48.75	48.75
	Total Core	75.00	75.00	75.00	75.00
Coat	CW	-	-	-	-
	HCO	25.00	25.00	25.00	25.00
	Total Coat	25.00	25.00	25.00	25.00
	Total Lipid	73.75	73.75	73.75	73.75
% Recovery ^s			>10	>10	>10
*Ingredients expressed as % of total.					
^s Percent recovery post encapsulation					

[0539] Final formulations will all yield on average at least a 10% recovery for anaerobic bacteria cells post-encapsulation, regardless of the lipid source used to create the core.

- [0540] Formulations will be packaged, stored at -20° C, and sampled at pre-determined time points to assess shelf-life following the protocol described in **Example 1**.
- [0541] Like for *M. elsdenii* in **Example 13**, average loss of the three batches over the first month of storage at 4° C or -20° C will be less than 0.3 log. Knowing that most losses occur within the first few months of storage, loss after 12-month of storage at -20° C will be less than 0.5 log. All lipid sources used to make the core will result in formulations that were stable enough for a commercial product.
- [0542] Formulations were then mixed with two high concentrate diets -- one low pH/high moisture diet (Table 2) and one high pH/high moisture diet (Table 3) -- to test in feed survival at 25° C and 52° C for up to 6 hours as described in **Example 1**.
- [0543] The formulation will provide protection to the anaerobic cells through mixing in feed with a decay of 25% or less for the first 4 hours at 25° C, regardless of the diet. In addition, anaerobic cells will be able to survive in the same diets when exposed to a higher temperature, 52° C, with a recovery of 30% or more after a 4-hour exposure. As expected, decay of anaerobic cells will be greater after 6-hour exposure regardless of diet and temperature. Time spent between feed mixing and distribution to the animal under normal conditions at a commercial feedlot is estimated to be less than 4 hours, showing that the formulations will provide sufficient protection for anaerobic cells to withstand mixing in feed and feed distribution at a commercial feedlot regardless of the lipid source used to create the core.
- [0544] Formulations will then be tested for rumen release following protocol described in **Example 1** to confirm that anaerobic cells are consistently released in the rumen after ingestion.
- [0545] Rumen release measured for the formulation(s) will fall within commercial criteria: 50% to be released within the 24-hour *in situ* incubation in rumen of steers fed high concentrate or high forage diets respectively regardless of the lipid source used to create the core.
- [0546] Formulations will further be tested for survival through micromachine and environmental exposure in the micromachine's bins following protocol described in **Example 1**.

[0547] On average, at least 70% of the anaerobic cells will survive the passage through the micromachine and at least 80% of the cells were recovered when left in the bins at 25° C, 30° C, or 37° C for to 14 hours, regardless of the lipid source used to create the core.

Example 22: Formulation pore size

[0548] Samples of core, formulation 8(X1), and formulation 16(X1) from prepared as in **Example 9** were sent to a third-party laboratory for porosity analysis. Results are shown in Table 40.

Table 40. Porosity Analysis

	Core	8(X1)	16(X1)
Median pore diameter (volume), μm^*	150.5	176.0	170.5
Median pore diameter (area), $\mu\text{m}^\&$	0.006	0.0053	0.0056
Porosity, %	40.0	34.8	39.8
*at 1.35 psia and 0.312 mL/g $\&$ at 32,029.40 psia and 18.875 m ² /g			

[0549] The median pore size for the formulation made of a two-step encapsulation was 170.5-176 μm indicating that the formulation tested can release the bacteria, *M. elsdenii* cells which range from 2.4 to 2.6 μm in size. In addition, those formulations had a percent porosity ranging from 35-40% further demonstrating that the formulations have the capacity to release the bacteria.

[0550] Finally, the small median pore diameter area, ranging from 0.0053 to 0.0056 μm is indicative of the presence of numerous capillaries in the formulations, rendering it highly porous and connected.

[0551] In comparison the core, obtained by mixing the freeze-dried *M. elsdenii* culture with maltodextrin and encapsulating it with hydrogenated palm oil (HPO) using spinning disk method, had lower median pore diameter but equivalent porosity and median pore diameter area.

Example 23: Alternative lipids for outer coat

[0552] *M. elsdenii* culture will be grown, cooled, concentrated and freeze-dried following methods described in WO 2018/144653 A1. An aliquot of the resulting freeze-dried culture will be mixed with Maltodextrin at a 1:5 ratio and encapsulated as described in Example 5. Formulations will be produced to have a small final particle size (212-710

µm) to increase surface area and maximize release post ingestion by cattle (rumen release). Encapsulated products will be analyzed for colony forming units (CFU) and will be compared to initial freeze-dried powder to determine percent recovery post-encapsulation following protocol described in Example 1.

[0553] Formulations using Hydrogenated Palm Oil (58°C to 62°C, 27 Stearine), Beeswax (62°C to 64°C), Hydrogenated Soybean Oil (66°C to 71°C, 17 Stearine), Paraffin (68.9°C to 72.8°C), Stearic Acid (69.3°C), and Candelilla (72.5°C) will be analyzed.

[0554] As in **Example 5**, *M. elsdenii* cells will be encapsulated using the lipids and the spinning disk method.

[0555] The formulations will be tested in feed using a low pH and high moisture diet to establish in feed survival following the procedure described in **Example 1**.

Example 24: Companion animals - Effect of daily *M. elsdenii* supplementation in companion animals (dogs)

[0556] Previous studies have demonstrated the clinical benefits of probiotics supplementation in companion animals including inhibiting proliferation of pathogenic bacteria, promoting the growth of beneficial bacteria, improving the intestinal barrier integrity, and modulating immune function. In addition, some of these benefits are linked to positive behavioral changes by alleviating stress and anxiety.

[0557] The goal of this experiment is to determine the impact of daily supplementation of companion animals with encapsulated *M. elsdenii* on healthy animals and on animals suffering from gastrointestinal disease.

[0558] Methods: At least six healthy dogs and six unhealthy dogs, 2–9 years of age, will be included in the study, with equal representation of male and female. Healthy control dogs will have no clinical signs of GI disease. Unhealthy dogs will have clinical signs of gastrointestinal disease (e.g., chronic enteropathy, exocrine pancreatic insufficiency, and/or diabetes) manifested by chronic diarrhea (stool score less than 3) for at least 3 weeks.

[0559] All dogs will be housed in individual outdoor kennels, with dirt surface that will be cleaned twice daily. Exercise will be provided daily but dogs will be segregated by groups (healthy vs. unhealthy).

[0560] All dogs will receive the same commercial maintenance diet for 30 days during the adaptation period. Dogs will consume the control diet (maintenance diet with no *M.*

elsdenii) or *M. elsdenii* diet (maintenance diet with *M. elsdenii* supplementation at 1×10^5 to 1×10^9 CFU/dog/day) for the next 30 days. Dogs will then be fed the maintenance diet alone (no *M. elsdenii* for any group; wash out period) for 30 days prior to a cross-over to the Control or *M. elsdenii* diet for 30 days. Dogs will be fed using electronic feeders, where fresh food is offered once a day with amounts calculated to maintain body weight. Study design is shown in Table 41.

Table 41. Study design.

	d0-d30 Adaptation period	d31-d60	d61-d90 Wash out period	d91-d120
Healthy Group	Maintenance diet	Maintenance diet	Maintenance diet	Maintenance diet + <i>M. e.</i>
Unhealthy Group	Maintenance diet	Maintenance diet + <i>M. e.</i>	Maintenance diet	Maintenance diet

[0561] Blood, urine, and fecal samples will be collected at the end of the adaptation period (d30) as well as approximately on days 15 and 30 of each subsequent period (day 45, 60, 75, 90, 105, and 120). Levels of metabolites and microbial composition will be assessed at the end of each 30-day feeding period (day 30, 60, 90, and 120). Body weight measurements and behavioral assessments using validated questionnaires will be performed weekly.

[0562] Stools will be collected within 30 min of defecation and scored from 1 to 5, where a score of 1 indicates >75% liquid and 5 is >80% firm. Feces will then be homogenized in a Mixer, aliquoted into vials, and frozen at -80°C until further analysis.

[0563] Blood chemistry (e.g., glucose, ketone, hemoglobin A1, and lactate), cortisol, and inflammatory cytokines will be analyzed using enzymatic colorimetric methods. Global plasma and fecal metabolite analyses will be performed using gas chromatography (for hydrophobic molecules) and liquid chromatography mass spectrometer (for hydrophilic molecules) platforms to identify and provide relative quantification of metabolites.

[0564] Serum chemistry will be performed to measure levels of triglycerides, creatinine, albumin, and cholesterol.

[0565] All fecal samples will be analyzed for fecal pH using a pH meter, and short chain fatty acids (SCFA) using gas chromatography, *Salmonella spp.* and *Campylobacter spp.* populations using selective culture methods.

- [0566] Total DNA will be extracted from thawed fecal samples and the fecal microbiome will be analyzed by performing 16S rRNA gene amplification of the V3-V4 regions and sequencing. The obtained raw sequences will be processed through a bioinformatics pipeline to eliminate sequences of insufficient quality and erroneous reads, remove chimeric sequences, and the final sequences will be compared against available public databases. The microbial profiles of the healthy and the unhealthy groups will be compared after the adaptation period. The effect of *M. elsdenii* supplementation on the abundance of the unique taxonomic operational units will be assessed by comparing changes in their abundance after the treatment period.
- [0567] Results: Unhealthy dogs will experience significant improvement in their stool qualities after they are fed *M. elsdenii*. This will be accompanied by a shift in their fecal microbial population. The fecal microflora of unhealthy dogs shifts towards the healthy group profile after they were fed the food supplemented with *M. elsdenii*. This includes an increase in the abundance of bacteria belonging to *Megasphaera*, *Blautia*, *Prevotella* and other beneficial bacteria and a decrease in the abundance of members of the family *Enterobacteriaceae*. In the absence of *M. elsdenii* supplementation, unhealthy dogs will have the highest fecal and blood lactate concentrations, suggesting that there is some accumulation of luminal lactate in GI diseases. Lactate accumulation may be due to poor carbohydrate digestion resulting in sugars being delivered to the colon, where the pH is decreased by organic acid production through fermentation, finally resulting in acid-resistant *Lactobacillus spp.* growing preferentially and lactate utilizing bacteria being inhibiting, therefore maintaining lactate production but decreasing lactate utilization. Unhealthy dogs receiving daily *M. elsdenii* supplementation will exhibit lower fecal and blood lactate concentrations than when they do not receive *M. elsdenii*.
- [0568] SCFA (e.g., butyrate, propionate, or acetate) will be greater in healthy animals versus unhealthy animals regardless of the diet received. In addition, *M. elsdenii* supplementation will increase the SCFA concentration in the animal when compared to its baseline without the supplementation. It is well known that SCFAs including acetate, propionate, and butyrate act as a preferred fuel source for the colonocytes and contribute to GIT health. *M. elsdenii* supplementation will also results in a change in SCFA ratios (butyrate, propionate, and acetate), which have inhibitory effect against pathogenic microorganisms (e.g. *Salmonella enterica* and *Escherichia coli*). Furthermore, increases

in butyrate and anti-inflammatory cytokines has been shown to have beneficial effects beyond the gastrointestinal tract, including reducing anxiety and stress.

[0569] Microbiome analysis will show differences in bacterial populations between the healthy and unhealthy groups. Supplementation with *M. elsdenii* will induce a shift in bacterial populations, especially in unhealthy dogs, resulting in a decrease in lactate producing bacteria, such as *bifidobacterium spp.*, *Enterococcus spp.*, and *Lactobacillus spp.*

[0570] Finally, analysis of the validated questionnaires will show that unhealthy dogs had reduced stress/anxiety after they consumed the food supplemented with *M. elsdenii*. The levels of stress hormones (i.e. Cortisol) and other stress/anxiety-related metabolites (i.e. inflammatory markers) will be significantly lower after they consumed the *M. elsdenii* supplemented food compared to the control.

[0571] *M. elsdenii* supplementation will have a beneficial effect on animal health and the effect will be even more important in animals suffering from gastrointestinal disease.

Example 25: Poultry - Evaluation of encapsulated *Megasphaera elsdenii* application in Broiler Chickens.

[0572] Goal: A pilot broiler performance study will be performed to evaluate the effects of daily *Megasphaera elsdenii* NCIMB 41125 administration on growth performance of broiler chicks.

[0573] Methods: Day-old broiler chicks (n=360) spray vaccinated with Coccivac-B on day 0 will be randomly allocated to 3 different treatments: (1) a negative control group receiving no *Megasphaera* (nCON), (2) a *M. elsdenii* group receiving *M. elsdenii* NCIMB 41125 on day 0 in feed (1×10^3 to 1×10^6 CFU/bird) daily, and (3) a positive control group (pCON) receiving no *M. elsdenii* NCIMB 41125, but receiving starter and grower feeds treated with BMD (50 g/t) and finisher feed treated with Stafac (20 g/t).

[0574] Each treatment will be represented by 4 cages containing 30 birds each. Diets provided to the birds will be as follows: starter feed from days 0-18, grower feed from days 19- 35, and finisher feed from day 36-39. Animal weights, feed consumption, and feed conversion will be recorded over the 39-day trial period.

[0575] Results: Overall mortality will not be different across treatments. Feed conversion for the *M. elsdenii* group will be significantly lower than either control group (nCON and

pCON). On the contrary, live weight for the *M. elsdenii* group will be increased in comparison to either control group (nCON and pCON).

Example 26: Poultry - Effect of *Megasphaera elsdenii* on *Salmonella* and *Campylobacter* Concentration and Prevalence in broiler chicks

- [0576] Goal: This study will be performed to evaluate the effects of daily *Megasphaera elsdenii* NCIMB 41125 administration on prevalence and concentration of *Salmonella* and *Campylobacter* in the cecum of broiler chicks.
- [0577] Methods: Day-old broiler chicks (n=192) will be randomly allocated to two different treatment groups: 1) a control group receiving no *M. elsdenii* NCIMB 41125, and 2) a daily *Megasphaera elsdenii* NCIMB 41125 group receiving encapsulated *M. elsdenii* daily in their diet (1×10^3 to 1×10^6 CFU/bird/day).
- [0578] Each of the two treatments will be represented by 16 cages containing 6 birds each. Animal weights, feed consumption, and feed conversion will be recorded over the 15-day trial period. After a 15-day feeding period, 2 animals per cage will be randomly selected, slaughtered, and ceca will be recovered to determine *Salmonella* and/or *Campylobacter* prevalence. Briefly, ceca will be retrieved, placed in bags, and stored on ice. Ceca will then be washed with 70% ethanol and manually massaged to extract content. One milliliter of the recovered content will be serially diluted in phosphate buffer saline solution (PBS) and plated onto respective selective agar. Selective agar plates will be incubated. Presumptive *Salmonella* and *Campylobacter* colonies will be counted and confirmed using rapid test kit. Additionally, one milliliter of cecum content sample will be added to 9 mL of respective selective enrichment media. If no detectable growth was observed on selective agar plates, enrichment will be plated onto selective agar plates again, incubated, and evaluated for the presence of *Salmonella* and *Campylobacter*. Samples with no growth with direct plating, but positive growth with enrichment method will be given an arbitrary count of 9 (1 below the theoretical detection limit) and samples with no growth in either direct plating or enrichment method will be given a count of 0.
- [0579] Birds receiving daily supplementation with encapsulated *M. elsdenii* will have lower cecal *Salmonella* and/or *Campylobacter* concentration as compared to the control group. The prevalence of *Salmonella* and/or *Campylobacter* in samples from the *M. elsdenii* group will also be decreased when compared to the control group.

Example 27: Poultry - Effect of *Megasphaera elsdenii* on Growth Performance and Cecal Characteristics of Broiler Chickens

- [0580] Experimental Design and Treatments
- [0581] Twenty-four replicates of two treatments in a randomized complete block design will be performed using Cobb 500 broiler chicks that will be one day old at the start of treatment. Treatments will be as follows: 1) a control group receiving no *M. elsdenii* and 2) a *M. elsdenii* group receiving daily supplementation of encapsulated *M. elsdenii* strain NCIMB 41125 in feed at 1×10^3 to 1×10^6 CFU/bird/day.
- [0582] The birds will be housed in 48 pens, with each pen containing 35 birds at the onset of the experiment (1,680 birds total). On the first day of the experiment, chicks will be allocated into groups of 35, and the weight of each group will be recorded. Groups of birds will be processed by block, with experimental treatments being assigned randomly within each block.
- [0583] To prevent cross contamination with treated birds, control birds will be handled only by designated personnel that will have no contact with treated birds and placed in designated carriers to be weighed and transferred to pens.
- [0584] Birds will have *ad libitum* access to fresh water. All diets will be fed in gravity feeders suspended in the center of the pen. Feed will be added as needed to ensure *ad libitum* access throughout the duration of the study. Birds will receive a starter diet (day 1-16), a grower diet (d17-30), and a finisher diet (d31-end of the study).
- [0585] Total feed consumption per pen for each phase (starter, grower, and finisher) will be calculated as: Feed added – feed recovered.
- [0586] Intake per bird per day will be calculated as: Total feed consumed ÷ [daily head count in pen x total days on feed]
- [0587] Pen weights will be recorded at the end of each feeding period (Starter, Grower, and Finisher).
- [0588] Each week (days 7, 14, 21, 28 and 35), 1 to 3 birds will be randomly selected from each pen and euthanized by cervical dislocation. Cecal contents (0.5 g) will be collected and analyzed for cecal pH, volatile fatty acids (VFA), quantification of *M. elsdenii* using quantitative real-time PCR.
- [0589] Birds will be slaughtered at 5-weeks of age to determine carcass measurements. Feed will be withheld approximately 4 h prior to slaughter. Five average sized birds will

be selected from each pen and placed into catch boxes for transport to the processing area. The 5 birds will be weighed by pen to determine live weight just prior to slaughter by stunning. The birds will be bled and plucked. The feet, head and shanks will be removed, and the carcasses eviscerated. The carcasses will then be weighed by pen to determine hot carcass yield.

[0590] Results: Broilers receiving *M. elsdenii* will demonstrate similar or improved feed intake, feed efficiency, and average daily gain when compared to control. Bird weights and mortalities will be unaffected or improved in the *M. elsdenii* treatment.

[0591] Cecal pH in birds receiving *M. elsdenii* will be significantly different compared to control birds.

[0592] Birds receiving *M. elsdenii* will have greater acetate, butyrate, caproate and total VFA concentrations in their cecal content compared to control birds. The acetate:propionate (A:P) ratio will also be greater in the cecal contents of birds treated with *M. elsdenii* compared to controls.

Example 28: Swine – Effect of daily *M. elsdenii* supplementation on growth performance and fecal characteristic of sow and their litters.

[0593] Goal: This study was designed to determine the effects of supplementation of sows and piglets with *M. elsdenii* on growth performance and fecal characteristics during the suckling and peri-weaning period.

[0594] Background: Weaning is a very stressful event during which piglets undergo abrupt changes in diet, environment, and social interactions. Consequently, weaning can result in major shifts in microbial population in the gastrointestinal tract and lead to dysbiosis.

[0595] Lactic acid producing bacterial species (e.g., *Lactobacillus*, *Bacillus*, *Enterococcus* or *Pediococcus spp.*) are commonly used probiotics in the swine industry. They have been shown to decrease pathogenic incidence and fecal shedding. One proposed mode of action is cross feeding between lactic acid producing bacteria and lactic acid utilizing bacteria (e.g., *M. elsdenii*) in the colon, resulting in the inhibition of dysentery causing pathogen, *Brachyspira hyodysenteriae*; however, the exact mechanism by which this occurs has not yet been established. One could hypothesize that the presence of lactic acid utilizing bacteria in the colon, could prevent the overgrowth of lactic acid producing bacteria in periods of stress and play an important role in preventing

dysbiosis, by maintaining colon pH and providing VFA that are necessary for enterocyte growth/proliferation intestinal mucosa health. As a result, piglets would experience improved pathogen resistance and nutrient absorption.

[0596] Sows often experience decreased intake during parturition, coupled with fat mobilization of body storage to meet energy requirements of lactation, which can result in excessive weight loss and have detrimental effects on subsequent reproductive performance. *M. elsdenii* supplementation in sows could increase VFA production, and therefore, provide additional butyrate that is the preferred energy source for colonocytes and known to help prevent mucosal atrophy, stimulate mucin secretion, and strengthen intestinal barrier. In time, *M. elsdenii* could improve intake by improving overall gut health and decreasing gastrointestinal (GI) upset, thereby stimulating appetite. *M. elsdenii* intervention could serve the dual purpose in sows of increasing feed intake, and therefore, energy availability, and stabilizing the microbiota of the GIT to prevent disease.

[0597] Methods: A total of 28 sows will be blocked by parity and allotted to 1 of 4 treatments organized in a split-plot design (Table 42) with the whole plot being the presence or absence of maternal supplementation of *Megasphaera elsdenii* and the subplot being the presence or absence of offspring supplementation of *Megasphaera elsdenii*.

Table 42. Split plot design.

Treatment	Maternal <i>M. elsdenii</i>	Offspring <i>M. elsdenii</i>
A	Yes	Yes
B	Yes	No
C	No	Yes
D	No	No

[0598] *M. elsdenii* supplementation will be provided daily in the form of encapsulated *M. elsdenii* at 1×10^5 to 1×10^9 CFU/sow and 1×10^3 to 1×10^6 CFU/piglet.

[0599] There will be 14 replications of the whole plot, 14 replications of the subplot, and 7 replications of the interactive plots. Treatments will be administered daily to the sows and/or piglets from the start of parturition.

[0600] Sows will all be fed the same common diet and managed according to normal procedures. At approximately day 21 of lactation, the piglets will be weaned and moved to a nursery facility. On the day of weaning, 1 piglet per litter will be sacrificed for total

cecal content collection. Piglets will be housed with 4-5 piglets per pen and piglets from the same treatment will be grouped in the same pens. Piglets will be fed the same common diet. Feed disappearance will be recorded and daily feed intake calculated.

- [0601] Sows will be weighed on day -3, -2, -1 prior to farrowing and on day 21 (at weaning). Piglets will be weighed on day 2, 21 (at weaning), 28, 35, 42, and 49.
- [0602] Fecal samples will be collected via rectal palpation from the sows on day -1, 7, 14, and 21 and from the piglets on day 21, 28, 35, 42, and 49.
- [0603] Fecal samples will be analyzed for pH using a pH meter and VFA concentrations using a gas chromatograph. Total cecal contents collected will be snap frozen in liquid nitrogen and stored at -80° C to await PCR analysis.
- [0604] Quantitative Real Time PCR analysis will be performed to quantify *M. elsdenii* in cecal samples at weaning.
- [0605] Results: Sows receiving *M. elsdenii* supplementation will have lesser weight loss and greater average daily feed intake in comparison to their counterpart not receiving any *M. elsdenii*. In addition, sows receiving *M. elsdenii* supplementation will present with higher cecal pH and increased VFA concentration (e.g., increase in propionate, butyrate, caproate and total VFA) compared to control animals.
- [0606] Piglets receiving *M. elsdenii* supplementation will have greater average daily gain, feed:gain and fecal pH compared to their counterparts not receiving *M. elsdenii*, regardless of the supplemental status of their mother. Like in sows, *M. elsdenii* treated piglets will have increased VFA concentrations, especially for butyrate and lower mortality.
- [0607] Overall, *M. elsdenii* will improve the gut environment, including the composition of end products of microbial fermentation, gut pH, and microbial colonization of the gut.

Example 29: Extended storage in micromachine bins of *M. elsdenii* encapsulated on a commercial scale in a two-step process using palm oil (HPO) and cottonseed oil (HCO).

- [0608] This study demonstrated the effectiveness of the two-step encapsulation with a hydrogenated palm oil core and a cottonseed oil coat in protecting *M. elsdenii* during extended storage in the micromachine bins when produced on a commercial scale.
- [0609] In this experiment, the final formulation composition was prepared as shown in Table 23 of **Example 13**. The three batches produced on a commercial scale (25 kg each)

were tested to mimic extended storage (for up to 48 hours) in micromachine bins at 25° C, 30° C, and 37° C (Table 43) prior to inclusion in the diet following protocol described in **Example 1**.

Table 43. Recovery following extended storage in micromachine bins with environmental exposure at 25° C, 30° C, or 37° C.

	25° C			30° C			37° C		
	24h	36h	48h	24h	36h	48h	24h	36h	48h
Average	88%	93%	81%	84%	87%	77%	74%	75%	56%

[0610] Cell loss due to environmental exposure in micromachine bins did not differ among batches and therefore only averages are presented in Table 43.

[0611] Increasing the temperature from 25° C to 37° C during the extended environmental exposure decreased cell recovery, with an additional 25% cell loss at 48 hours. However, minimal differences (4%) were observed when comparing 25° C and 30° C incubation at 48 hours. These data demonstrate that the formulation can withstand storage in the bins for an extended period of time prior to delivery through the micromachine, while still providing >56% or more of live cells.

Example 30: Production of anaerobic encapsulated cells in a two-step process using spray dried cells.

[0612] This example demonstrates the applicability and effectiveness of a two-step encapsulation with a hydrogenated palm oil core and a cottonseed oil coat for *M. elsdenii* (see **Example 18**) produced by spray drying (WO 2024/026095 A1).

[0613] *M. elsdenii* were grown, cooled, concentrated and spray dried following method described in WO 2024/026095 A1. The resulting powders were encapsulated with hydrogenated palm oil (HPO) mixed with two amounts of carrier (maltodextrin; 13.23% and 18.75%), using spinning disks to form a core. The core was then coated with hydrogenated cottonseed oil (HCO) to obtain a final product with particle size ranging from 212 to 710 µm. The final formulations' compositions are listed in Table 44. Formulations were produced and tested for recovery post-encapsulation (Table 44), shelf-life, infeed survival, and passage through a micromachine following protocols described in **Example 1**.

[0614] Rumen release will be tested using a Daisy rumen model. Briefly, rumen fluid collected from a steer fed a high concentrate diet, recently slaughtered, will be strained and placed in 400 mL aliquots into jars containing 1600 mL of saliva buffer. Pre-weighed filter bags, containing 0.5 g of the formulations and a lead fishing weight (to weight the bag down), will be added to the jar. Contents of the jar will be mixed and incubated at 39° C under anaerobic conditions for 24 hours. At the end of the incubation time, the digestion jars will be removed from the incubator and filter bags will be collected, dried, and weighed to determine dry matter disappearance.

Table 44. Formulations composition and *M. elsdenii* cell recovery after encapsulation process.

Portion	Ingredient*	Spray dried cells	
		Trt1	Trt3
Core	Dried <i>M. elsdenii</i> cells	13.02	7.50
	Carrier (i.e., Maltodextrin)	13.23	18.75
	HPO	48.75	48.75
	Total Core	75.00	75.00
Coat	CW	-	-
	HCO	25.00	25.00
	Total Coat	25.00	25.00
	Total Lipid	73.75	73.75
% Recovery^s		36	34

*Ingredients expressed as % of total.

^sPercent recovery post encapsulation

[0615] Regardless of the amount of carrier used, final formulation yielded on average at least a 30% recovery for *M. elsdenii* cell post encapsulation.

[0616] Formulations were packaged, stored at 4° C and -20° C and sampled at pre-determined time point to assess shelf-life following protocol described in **Example 1**.

[0617] Like for *M. elsdenii* in **Example 13**, average loss over the first month of storage at 4° C or -20° C were less than 0.3 log regardless of the amount of carrier (maltodextrin) used. Knowing that most losses occur within the first few months of storage, loss after 12-month of storage at -20° C will be less than 0.5 log.

[0618] The formulations were then mixed with a high concentrate diet with low pH and high moisture diet (Table 2) to test in feed survival at 25° C and 52° C for up to 4 hours as described in **Example 1**.

- [0619] The formulations provided protection to *M. elsdenii* cells through mixing in feed with a recovery of 47% or more for the first 4 hours at 25° C, regardless of the amount of maltodextrin used in the formulation. In addition, *M. elsdenii* cells were able to survive in the same diet when exposed to a higher temperature, 52° C, with a recovery of 18% or more after a 4-hour exposure. Time spent between feed mixing and distribution to the animal under normal conditions at a commercial feedlot is estimated to be less than 4 hours, showing that the formulation will provide sufficient protection for *M. elsdenii* cells to withstand mixing in feed and feed distribution at a commercial feedlot, regardless of the amount of carrier used.
- [0620] On average, at least 55% of the *M. elsdenii* cells survived the passage through the micromachine.
- [0621] Formulations will also be tested for rumen release following Daisy rumen model protocol listed above to confirm that anaerobic cells are consistently released in the rumen after ingestion.
- [0622] Since spray dried *M. elsdenii* encapsulated in a two-step process showed similar results as observed in freeze dried *M. elsdenii* encapsulated using the same process for cell survival, survival in feed and through micromachine, it is expected that the rumen release will be no different. Rumen release measured for the formulation(s) will fall within commercial criteria: 50% to be released within the 24-hour *in situ* incubation in rumen fluid of steers fed high concentrate diet regardless of the amount of maltodextrin used.
- [0623] These data demonstrate that spray dried *M. elsdenii* cells can be encapsulated in a two-step process and produce live cells that can withstand storage, mixing in feed, passage through the micromachine and still be released in the rumen. These results are not different from the results observed when the *M. elsdenii* cells are freeze-dried and then encapsulated in the same manner (**Example 13**), therefore the two-step encapsulation process is successful at protecting *M. elsdenii* cells regardless of the drying method used prior to encapsulation.

Example 31 – Effect of *M. elsdenii* oral drench on reticulorumen pH dynamics in lactating dairy cows under subacute ruminal acidosis challenge

- [0624] Subacute ruminal acidosis (SARA) is an important disorder in dairy cattle with economic and animal welfare implications. A preventive strategy for SARA control is oral drenching of probiotics, such as *Megasphaera elsdenii*.
- [0625] The aim of this study was to evaluate the effects of a *M. elsdenii* oral drench on reticulorumen pH, milk yield and components, and feeding behavior of lactating cows under a SARA challenge.
- [0626] This study consisted of two crossover trials with 8 cows each to determine the efficacy of drenching live cultures of *M. elsdenii* NCIMB 41125. The experimental period of each trial lasted 8 days with a 4 week washout interval between periods. Each animal was assigned to one individual feeder using each cow's radio frequency identification (RFID) tag to operate and record the feed intake. Each animal ate from their assigned bin until the end of the experimental period. The first 3 days of each period were considered baseline days. On day 4 of the period, feed delivered was reduced by half, based upon individual mean dry matter intake (DMI) during the baseline period. On day 5 of the period, all cows received a challenge diet, which was rich in highly fermentable carbohydrates, offered to the cows for 2 hours to induce SARA. The challenge mix consisted of 2 kg of rolled barley, 2 kg of ground wheat and 0.9 kg of molasses that was combined with 4.3 kg of total mixed ration (TMR). Orts were weighed and replaced with regular diet offered *ad libitum*. The last 3 days of each experimental period were considered recovery days.
- [0627] The difference between each trial was the administration time of *M. elsdenii* drench (100 mL of LactiproNXT[®] containing approximately 2×10^8 CFU/mL), where in Trial 1 it was delivered 4 days before (PRO-4) and in Trial 2 it was delivered the day before (PRO-1) the SARA challenge. Cows were randomly assigned to either a treatment (PRO-4 or PRO-1) or control (CON-4 or CON-1) drench, and reversed on the subsequent period.
- [0628] Milk yield and components, dry matter intake (DMI) and feeding behavior (time spent feeding and number of visits to the feeder), and reticulorumen pH were recorded continuously during the entire experimental period. Total mixed ration samples were collected during the baseline days and analyzed. Samples for nutrient and dry matter

(DM) analysis were oven dried at 55 °C for 48 hours. Dried samples were ground to pass through a 1-mm screen and analyzed for acid detergent fiber (ADF) (AOAC International, 2000: method 973.18), neutral detergent fiber (NDF) with heat-stable α -amylase and sodium sulphite (Van Soest et al., 1991), and crude protein (CP) (N x 6.25; AOAC International 2000: method 990.03; Leco FP-528 Nitrogen Analyzer, Leco, St. Joseph, MI).

[0629] During Trial 1, PRO-4 cows produced more milk ($P < 0.01$), with greater protein percentage ($P=0.03$) and lesser fat to protein ratio ($P=0.01$) compared with control cows. PRO-4 cows also had greater overall reticulorumen pH and experienced shorter and less intense acidosis ($P < 0.05$) and greater DMI ($P=0.04$) compared with control cows. Feeding behavior was not affected by treatment in Trial 1 ($P > 0.10$).

[0630] In Trial 2, only feeding time was affected by the treatment, with PRO-1 cows spending more minutes per day feeding ($P < 0.01$) compared to control cows.

[0631] The results indicate potential benefits of a *M. elsdenii* drench on rumen pH dynamics, acidosis resilience, and possibly milk production and feed intake (Table 45). However, it appears that the time of drench administration might influence drench efficacy. Stabilization of the *M. elsdenii* for a daily administration in feed would facilitate timing of the treatment and ensure maximum efficiency of the product.

Table 45. Least square means ($\pm$ SEM) of reticulorumen pH dynamics, DMI, feeding behavior, and milk yield and composition for dairy cows (n=8) drenched with distilled water (control) or *Megasphaera elsdenii* 4 days (Trial 1) and 1 day (Trial 2) before an acidosis challenge.

	PRO-4*			PRO-1#		
	Treatments			Treatments		
	Control	M. elsdenii	SEM	P-value	Control	M. elsdenii
	Control	M. elsdenii	SEM	P-value	Control	M. elsdenii
Reticulorumen pH Dynamics						
Mean reticulorumen pH average	6.11	6.23	0.05	<0.01	6.24	6.21
Area below pH 5.8 (min × pH/d)	81.38	20.85	27.35	0.01	21.54	36.26
Area below pH 5.6 (min × pH/d)	37.31	6.77	13.89	0.03	19.12	17.01
Time below pH 5.8 (h/d)	4.68	1.82	1.42	<0.01	2.61	3.20
Time below pH 5.6 (h/d)	2.65	0.64	0.88	0.01	1.66	1.31
DMI and Feeding Behavior						
DMI (kg/d)	21.43	23.50	1.45	0.04	22.80	22.88
Visits to the feeder (visits/d)	27.99	29.34	1.65	0.44	37.42	35.98
Time spent feeding (min/d)	191.65	200.62	27.72	0.59	282.66	378.19
Milk yield and Components						
Milk yield (kg/d)	26.07	28.75	1.62	0.01	34.26	34.97
Milk fat (%)	4.09	3.97	0.08	0.06	3.78	3.82
Milk protein (%)	3.19	3.28	0.15	0.02	3.21	3.22
Fat to protein ratio	1.30	1.22	0.06	0.01	1.18	1.19
*M. elsdenii 4 days before an acidosis challenge						
#M. elsdenii 1 day before an acidosis challenge						

**M. elsdenii* 4 days before an acidosis challenge

[#]*M. elsdenii* 1 day before an acidosis challenge

Example 32 – Effect of daily administration of encapsulated *M. elsdenii* on reticulorumen pH dynamics in lactating dairy cows subacute ruminal acidosis (SARA) challenge.

- [0632] The objective of this study will be to evaluate the effect of daily administration of encapsulated *M. elsdenii* mixed in the feed at 1×10^7 , 1×10^8 , or 1×10^9 CFU/head/day on reticulorumen pH dynamics, milk yield, milk composition, feed intake, feeding behavior and rumen fluid composition in lactating dairy cows under acidotic challenge.
- [0633] Thirty-two Holstein multiparous cows, beginning around 90 days in milk (DIM), will be used in this experiment. Cows will be sorted into four blocks and assigned randomly to one of four treatments (8 cows per treatment):
- **Control (Control)** - no *M. elsdenii* administered.
 - **10^7 (Low)** - encapsulated *M. elsdenii* will be administered daily to deliver an estimated 2×10^7 CFU per head daily.
 - **10^8 (Medium)** - encapsulated *M. elsdenii* will be administered daily to deliver an estimated 2×10^8 CFU per head daily.
 - **10^9 (High)** - encapsulated *M. elsdenii* will be administered daily to deliver an estimated 2×10^9 CFU per head daily.
- [0634] The experimental period will consist of a 10 day acclimation period, a 1 day challenge period, and a 10 day recovery period (22 day in total length).
- [0635] Encapsulated *M. elsdenii*: Encapsulated products to be used in this experiment will be prepared as described in **Example 13**, with Table 46 showing the final composition. Encapsulated *M. elsdenii* will then be further mixed with varying amounts of a carrier (maltodextrin) to meet the CFU/50 mg/head/day.

Table 46. Formulation compositions

Portion	Ingredient*	Formulation
Core	Freeze dried <i>M. elsdenii</i> cells	4.35
	Carrier (i.e. Maltodextrin)	21.90
	HPO	48.75
	Total Core	75.00
Coat	CW	-
	HCO	25.00
	Total Coat	25.00
	Total Lipid	73.75

*Ingredients expressed as % of total.

- [0636] The treatments will be top dressed in a small portion of the TMR and will be administered in each bunk prior to feeding. Once the bunk is clean, the animal will receive the remaining of the TMR. The treatment delivery will begin on day 0 of the adaptation period and fed through to day 22 of the recovery period.
- [0637] On day 10, cows will undergo a 24-hour feed restriction in which they will only receive 50% of their previous 3 day average intake. On day 11, cows will be offered 2 kg of rolled barley + 2 kg ground wheat + 0.9 kg molasses mixed with 4.3 kg of their regular TMR mixture for 2 hours. After which, the orts will be weighed and subsequently replaced with the remaining of the TMR ration.
- [0638] Cows will be housed in a tie stall venue consisting of pine-bedded tie stalls (1.2 x 2.4 m). Cows will be assigned to one bed and tied to a collar. Cows will have access to feed via a white feeding tub and *ad libitum* water via Ritchie Water Troughs for the duration of the experiment outside of the challenge period. Cows will be let out 3 times daily to travel to/from the parlor.
- [0639] The milk cow ration will be fed once daily at approximately 0900 hour as a TMR. Total feed delivered to each individual cow and total refusals will be recorded each day to calculate daily DMI.
- [0640] On the day prior to the start of the experiment (-1 day), cows will be equipped with an ear based rumination sensor (Cow Manager) to measure activity and eating behaviors, as well as with a SmaXtec rumen pH bolus to measure rumen pH every 10 minutes.
- [0641] Cows will be milked three times daily, and milk yield will be recorded for individual cows at each milking session as well as body weight, throughout the duration of the trial. Milk samples will be collected from individual cows from 3 consecutive milkings at the following intervals; day 1-day 3, day 7-day 9, day 11-day 13, day 20-day 22. Samples from consecutive days will be composited based on milk yield, preserved and be analyzed for true protein, fat, lactose, solids-not-fat (SNF), and milk urea nitrogen (MUN) concentrations, somatic cell counts (SCC) and milk fatty acid profiles.
- [0642] Rumen fluid will be extracted via the Rumen-Mate pump, 6 hours post feeding (or challenge), on the day prior to the start of the experiment (-1 day), day 5, day 9 (day before the feed restriction), day 11 (after challenge), day 12, day 13, day 16, day 23, and

day 30. The pH of the rumen fluid sample will be measured immediately after sampling using a portable pH meter (Ohaus ST20 pH Pen Meter). The rumen fluid samples will then be filtered through four layers of cheesecloth. Fifty milliliter aliquots of the resulting fluid will be placed in pre-labelled falcon tubes (2 falcon tubes/animal/sampling) and immediately frozen at -20° C for subsequent VFA and lactate analysis. Another aliquot will be placed in SafeCollect™ collection tubes (DNA/RNA Shield, Cat # R1211, Zymo Research, California), thoroughly mixed and immediately frozen at -20° C for subsequent qPCR analysis.

- [0643] On days 11, 12, and 13, a subsample of the rumen fluid collected will be used to perform lactate utilization (disappearance) assay as previously described in **Example 17**.
- [0644] Cows receiving encapsulated *M. elsdenii* will have higher overall reticulorumen pH and experience shorter and less intense acidosis bout. In addition, those cows will have greater DMI and will spend more time eating compared with control cows. Lactate utilization (disappearance) measured *in vitro* will also be more efficient for the cows receiving encapsulated *M. elsdenii*.
- [0645] Compared to Example 31 that follows a similar experimental design but testing a one-time *M. elsdenii* drench, this experiment will show the superiority of a daily administration of the encapsulated *M. elsdenii* and its ability to further improve rumen pH dynamics, acidosis resilience, and possibly milk production and feed intake in dairy cows.

Example 33 – Effect of daily administration of encapsulated *M. elsdenii* on early lactation performance of dairy cows

- [0646] Dairy cows, much like beef cattle, are transitioned from a low energy diet during the dry period to a high energy diet during close-up and early lactation periods. Direct fed microbials, such as *M. elsdenii*, have been considered as an option for decreasing the occurrence of metabolic disorder during that period as well as a means to accelerate the transition, easing the dairy management, and potentially resulting in operational savings.
- [0647] The objective of this experiment will be to determine the effect of daily administration of encapsulated *M. elsdenii* in dairy cow feed during the transition period (close up and early lactation) on rumen pH, milk performance, and overall health.
- [0648] A total of eighty (80) cows calving into their second lactation or greater will be used from 21 days prepartum to 56 days postpartum. Four rounds of twenty cows will be moved to the BioControl pen beginning 28 days prepartum and allowed 7 days to train to

an individual feeding station. Cows will be used in a complete randomized block design and will be randomly assigned to one of four treatments:

- **Control (Control)** - no *M. elsdenii* administered.
- **Low** - encapsulated *M. elsdenii* will be administered daily to deliver an estimated 0.5 X of *M. elsdenii* per head daily.
- **Medium** - encapsulated *M. elsdenii* will be administered daily to deliver an estimated 1 X of *M. elsdenii* per head daily.
- **High** - encapsulated *M. elsdenii* will be administered daily to deliver an estimated 2 X of *M. elsdenii* per head daily.

[0649] The amount of *M. elsdenii* in each group will be determined based on the results from **Example 32**. The treatments will be delivered to each group beginning 21 days prior to calving and continue for the first 21 days of lactation.

[0650] Cows will be housed in a large pen consisting of twenty-four sand-bedded freestalls (1.2 x 2.4 m) and have *ad libitum* access to feed via the BioControl Feeding Stations and water via Ritchie Water Trough.

[0651] All cows will receive two basal rations, a close-up diet formulated for the dry period and a lactation diet. Both rations will be fed once daily at approximately 0830 hours as a total mixed ration (TMR). TMR (before treatment delivery) and individual ingredients will be collected weekly throughout the study and stored at -20 °C until analysis.

[0652] Daily DMI and eating behaviors will be collected using the BioControl feeding stations and analyzed on an individual basis pre and postpartum to determine if treatments had any impact on eating behaviors such as meal intervals, eating and feeding rates, and visits.

[0653] Upon entry in the study, cows will be weighed, receive a SmaXtec rumen pH bolus to record rumen pH every 10 minutes and will be fitted with CowManager® tags in the ear (Agis, Harmelen, Netherlands) to monitor rumination and eating behavior for the entire duration of the study.

[0654] Rumen fluid will be extracted via the Rumen-Mate pump, 6 hours post feeding, at the start of the experiment (-day 21), day 3, day 28 and day 56 relative to calving. The pH of the rumen fluid sample will be measured immediately after sampling using a portable pH meter (Ohaus ST20 pH Pen Meter). The rumen fluid samples will then be filtered through four layers of cheesecloth. Fifty milliliter aliquots of the resulting fluid will be

placed in pre-labeled falcon tubes (2 falcon tubes/animal/sampling) and immediately frozen at -20 °C for subsequent VFA and lactate analysis. Another aliquot will be placed in SafeCollect™ collection tubes (DNA/RNA Shield, Cat # R1211, Zymo Research, California). The tubes will be thoroughly mixed and immediately frozen at -20 °C for subsequent qPCR analysis for *M. elsdenii* population.

[0655] After calving, cows will be milked three times daily, and milk yield will be recorded for individual cows at each milking session throughout the duration of the trial. Milk samples will be collected from individual cows on a weekly basis and analyzed for true protein, fat, lactose, SNF, MUN concentrations, and milk fatty acid profile. For each cow, yield of milk components will be calculated by multiplying component concentration by test day and milk yield for the session's samplings. Individual cow body weights will be recorded after each milking session.

[0656] Cow events including transitional issues, medical health treatments and non-medical observations will be recorded. At the end of the study (day 56), cows will be returned to the commercial herd. Lactation and service data will be monitored until next lactation to determine effect of the daily encapsulated *M. elsdenii* administration on total milk production, cull rate, and reproduction.

[0657] Cows receiving daily supplementation of encapsulated *M. elsdenii* during the close-up and early lactation period will produce more milk, with greater protein percentage, and lesser fat to protein ratio compared with control cows and regardless of the dose used (Low, Medium, or High). The *M. elsdenii* animals likely will reach peak milk sooner and produce more milk at peak milk. Cows receiving daily encapsulated *M. elsdenii* supplementation will also have greater overall reticulorumen pH and experienced shorter and less intense acidosis bout with ruminal pH below 5.6 and 5.8 compared to the control animals. Ruminal fluid composition will differ with an increase in propionate and butyrate in the animals receiving daily supplementation with encapsulated *M. elsdenii*. In addition, *M. elsdenii* groups will have greater DMI and increased feeding behavior compared to the animals not receiving any *M. elsdenii*. Finally, in comparison to the control group, the number of cows culled from the *M. elsdenii* group will be lowered, the time to conception will be shorten and the pregnancy rate will be increased.

[0658] These results will support the tendency noted in previous dairy studies using a one-time *M. elsdenii* drench and confirm that a daily administration of an encapsulated *M.*

elsdenii during close-up and early lactation is able to maintain benefit over the course of the full lactation period.

WHAT IS CLAIMED IS:

1. A composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the *Megasphaera elsdenii* cells are viable in the composition.
2. A composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a feed or feed additive.
3. The composition of claim 2, wherein the *Megasphaera elsdenii* cells in the composition are viable for up to 48 hours at 25° C and pH 7.0 after being added to the feed or feed additive.
4. A composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein administration of the composition to a ruminant results in viable *Megasphaera elsdenii* cells being released in the rumen.
5. A composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a temperature of at least 40° C to 60° C at pH 7.0 for 4-18 hours.
6. A composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more

lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a pH between 3-7 at 25° C for up to 48 hours.

7. A composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the *Megasphaera elsdenii* cells in the composition are viable when the composition is exposed to a pH between 3-7 and a temperature between 25° C to 60° C for up to 48 hours.
8. A composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being processed through a micromachine system.
9. A composition comprising (a) a core comprising (i) *Megasphaera elsdenii* cells; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for up to 48 hours.
10. The composition of claim 9, wherein at least 10% of the *Megasphaera elsdenii* cells in the composition are viable after being added to a microbin of a micromachine for about 14 hours to about 48 hours.
11. The composition of any one of claims 1-10, wherein the particle size of the *Megasphaera elsdenii* cells in the composition are less than about 1600 µm.
12. The composition of any one of claims 1-11, wherein the particle size of the *Megasphaera elsdenii* cells in the composition are less than about 400 µm.

13. A composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are viable in the composition.
14. A composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a feed or feed additive.
15. The composition of claim 14, wherein the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable for up to 48 hours at 25° C and pH 7.0 after being added to the feed or feed additive.
16. A composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein the administration of the composition to a ruminant results in viable bacterial cells being released in the rumen.
17. A composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a temperature of 40° C to 60° C at pH 7.0 for 4-18 hours.
18. A composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids,

and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a pH between 3-7 at 25° C for up to 48 hours.

19. A composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable when the composition is exposed to a pH between 3-7 and a temperature between 25° C to 60° C for up to 48 hours.
20. A composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being processed through a micromachine system.
21. A composition comprising (a) a core comprising (i) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state; (ii) at least one carrier; and (iii) one or more lipids, and (b) at least one layer of one or more lipids, wherein the core is coated by the layer of one or more lipids, and wherein at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine for up to 48 hours.
22. The composition of claim 21, wherein at least 10% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are viable after being added to a microbin of a micromachine for about 14 hours to about 48 hours.

23. The composition of any one of claims 13-22, wherein the particle size of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are less than about 1600 μm .
24. The composition of any one of claims 13-23, wherein the particle size of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state in the composition are less than about 400 μm .
25. The composition of any one of claims 13-24, wherein at least 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99% of the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are viable in the composition.
26. The composition of any one of claims 13-25, wherein the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are dried.
27. The composition of any one of claims 13-26, wherein the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are dried by spray drying, electrospray drying, vacuum drying, jet drying, freeze-drying, or combinations thereof.
28. The composition of any one of claims 13-27, wherein the composition comprises from about 0.1% to about 15% (w/w) anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state.
29. The composition of any one of claims 13-28, wherein the composition comprises anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state from about 1×10^4 to about 1×10^{10} CFU/g.
30. The composition of any one of claims 13-29, wherein the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state are selected from the group consisting of *Bifidobacterium breve*, *Lactobacillus plantarum*, *Bifidobacterium animalis subsp. lactis*, *Pediococcus acidilactici*, *Lactobacillus casei*, *Megasphaera elsdenii*, *Fibrobacter*

succinogenes, *Butyrivibrio fibrisolvens*, *Ruminococcus flavefaciens*, *Blautia obeum*, *Clostridium butyricum*, *Akkermansia muciniphila* and combinations thereof.

31. The composition of any one of the previous claims, wherein the composition is a granule, capsule, minicapsule, microcapsule, tablet, minitab, or microtablet.
32. The composition of any one of the previous claims, wherein the composition has a moisture content of about 5% (w/w) or less.
33. The composition of any one of the previous claims, wherein the one or more lipids in the core is selected from the group consisting of animal oils or fats, vegetable oils or fats, triglycerides, free fatty acids, animal waxes, beeswax, lanolin, shell wax, Chinese insect wax, vegetable waxes, carnauba wax, candelilla wax, bayberry wax, sugarcane wax, mineral waxes, synthetic waxes, natural and synthetic resins, and mixtures thereof.
34. The composition of any one of the previous claims, wherein the one or more lipids in the core is an animal fat or oil and/or a plant fat or oil.
35. The composition of claim 34, wherein the plant fat or oil is selected from the group consisting of canola oil, cottonseed oil, hydrogenated cottonseed oil, peanut oil, corn oil, olive oil, soybean oil, hydrogenated soybean oil, sunflower oil, safflower oil, coconut oil, palm oil, hydrogenated palm oil, linseed oil, tung oil, castor oil and rapeseed oil.
36. The composition of claim 34, wherein the plant fat or oil is hydrogenated palm oil.
37. The composition of claim 33, wherein the free fatty acid is myristic acid, lauric acid, or stearic acid, or combinations thereof.
38. The composition of any one of the previous claims, wherein the one or more lipids in the core has a melting point of about 40° C to about 85° C.

39. The composition of any one of the previous claims, wherein the one or more lipids in the core has a melting point of about 55° C to about 75° C.
40. The composition of any one of the previous claims, wherein the one or more lipids coating the core is selected from the group consisting of animal oils or fats, vegetable oils or fats, triglycerides, free fatty acids, animal waxes, beeswax, lanolin, shell wax, Chinese insect wax, vegetable waxes, carnauba wax, candelilla wax, bayberry wax, sugarcane wax, mineral waxes, synthetic waxes, natural and synthetic resins, and mixtures thereof.
41. The composition of any one of the previous claims, wherein the one or more lipids coating the core is an animal fat or oil and/or a plant fat or oil.
42. The composition of claim 41, wherein the plant fat or oil is selected from the group consisting of cottonseed oil, hydrogenated cottonseed oil, peanut oil, corn oil, olive oil, soybean oil, hydrogenated soybean oil, sunflower oil, safflower oil, coconut oil, palm oil, hydrogenated palm oil, linseed oil, tung oil, castor oil and rapeseed oil.
43. The composition of claim 41, wherein the plant fat or oil is hydrogenated palm oil or hydrogenated cottonseed oil.
44. The composition of claim 40, wherein the free fatty acid is myristic acid, lauric acid, or stearic acid.
45. The composition of any one of the previous claims, wherein the one or more lipids coating the core has a melting point of about 55° C to about 80° C.
46. The composition of any one of the previous claims, wherein the one or more lipids coating the core has a melting point of about 55° C to about 75° C.
47. The composition of any one of the previous claims, wherein at least one carrier comprises maltodextrin, sucrose, starch, cellulose, clay, biochar, lignin-derivatives, sugar alcohols, or combinations thereof.

48. The composition of any one of the previous claims, wherein the composition comprises a total of about 10% to about 99% (w/w) lipids.
49. The composition of any one of the previous claims, wherein the composition comprises a total of about 70% to about 80% (w/w) lipids.
50. The composition of any one of the previous claims, wherein the composition comprises from about 10% to about 30% (w/w) of at least one carrier.
51. The composition of any one of the previous claims, wherein the composition comprises from about 15% to about 25% (w/w) of at least one carrier.
52. The composition of any one of the previous claims, wherein the composition is from about 0.1 mm to about 3 mm diameter in size.
53. The composition of any one of the previous claims, wherein the composition is from about 0.2 mm to about 0.6 mm diameter in size.
54. The composition of any one of the previous claims, wherein the composition is from about 0.2 mm to about 0.4 mm diameter in size.
55. The composition of any one of the previous claims, wherein the core comprises from about 1% to about 99% of the composition.
56. The composition of any one of the previous claims, wherein the core comprises from about 10% to about 90% of the composition.
57. The composition of any one of the previous claims, wherein the core comprises from about 25% to about 80% of the composition.

58. The composition of any one of the previous claims, wherein the one or more lipids coating the core comprises from about 1% to about 99% of the composition.
59. The composition of any one of the previous claims, wherein the one or more lipids coating the core comprises from about 5% to about 75% of the composition.
60. The composition of any one of the previous claims, wherein the composition has a density from about 0.6 g/mL to about 1.2 g/mL.
61. The composition of any one of the previous claims, wherein the composition has a porosity from about 10% to about 60%.
62. The composition of any one of claims 1-12 and 31-61, wherein at least 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99% of the *Megasphaera elsdenii* cells are viable in the composition.
63. The composition of any one of claims 1-12 and 31-62, wherein the *Megasphaera elsdenii* cells are dried.
64. The composition of any one of claims 1-12 and 31-63, wherein the *Megasphaera elsdenii* cells are dried by spray drying, electrospray drying, vacuum drying, jet drying, freeze-drying, or combinations thereof.
65. The composition of any one of claims 1-12 and 31-64, wherein the composition comprises from about 0.1% to about 15% (w/w) *Megasphaera elsdenii* cells.
66. The composition of any one of claims 1-12 and 31-65, wherein the composition comprises *Megasphaera elsdenii* cells from about 1×10^4 to about 1×10^{10} CFU/g.

67. The composition of claims 13-62, wherein the anaerobic bacterial cells or anaerobic bacterial cells in a vegetative state comprises from about 1×10^4 to about 1×10^{10} CFU/g in the composition.
68. The composition of any one of claims 1-67, wherein the composition further comprises one or more of antibiotics, antimicrobials, anti-coccidials, antiparasitics, sulfonamides, hormones, anti-bloat compounds, adrenergic receptor modulators, a phage, a prebiotic, probiotics, enzymes, essential oils, and/or a carbohydrate immune stimulant.
69. A feed additive composition comprising the composition of any one of claims 1-68.
70. The feed additive composition of claim 69, wherein the feed additive composition is a powder, granulate, particulate, pellet, cake, liquid, solid, suspension, emulsion, gel, or combinations thereof.
71. A feed comprising the composition of any one of claims 1-67 or the feed additive composition of claims 69 or 70.
72. The feed of claim 71, further comprising an animal protein, a vegetable protein, corn, soybean meal, corn dried distillers grains with solubles (cDDGS), wheat, wheat proteins, gluten, wheat by products, wheat bran, wheat dried distillers grains with solubles (wDDGS), corn by products including corn gluten meal, barley, oat, rye, triticale, full fat soy, animal by-product meals, an alcohol-soluble protein, a zein, a maize zein, a kafirin, rice, paddy rice, extruded paddy rice, a protein from oil seeds, or a combination thereof.
73. The feed of claim 72, wherein the animal protein or vegetable protein is selected from the group consisting of one or more of a gliadin or an immunogenic fragment of a gliadin, a beta-casein, a beta-lactoglobulin, glycinin, beta-conglycinin, cruciferin, napin, hordeins, keratins, feather or hair meals, collagen, whey protein, fish protein, fish meals, meat protein, egg protein, soy protein and grain protein.

74. The feed of claim 72, wherein the protein from oil seeds is selected from the group consisting of soybean seed proteins, sunflower seed proteins, rapeseed proteins, canola seed proteins and combinations thereof.
75. A premix comprising a) the composition of any one of claims 1-68 or the feed additive composition of any one of claims 69 or 70; and b) at least one mineral and/or at least one vitamin.
76. A kit comprising a i) the composition of any one of claims 1-68; ii) the feed additive composition of any one of claims 69 or 70; iii) the feed of any one of claims 71-74; and/or iv) the premix of claim 75; and b) instructions for formulating and/or administering to a subject.
77. A method for treating or preventing a condition or disorder associated with lactic acid production in the gastrointestinal tract of a subject, comprising administering to the subject an effective amount of the composition of any one of claims 1-68, the feed additive composition of any one of claims 69 or 70, or the feed of any one of claims 71-74.
78. The method of claim 77, wherein the condition or disorder is acidosis.
79. The method of claim 77, wherein the condition or disorder is ruminal acidosis.
80. The method of claim 77, wherein the condition or disorder is respiratory disease.
81. The method of claim 77, wherein the condition or disorder is laminitis.
82. A method for preventing or decreasing the growth of an opportunistic microorganism in the gastrointestinal tract of a subject, comprising administering to the animal an effective amount of the composition of any one of claims 1-68, the feed additive composition of any one of claims 69 or 70, or the feed of any one of claims 71-74.

83. The method of claim 82, wherein the opportunistic microorganism is pathogenic.
84. The method of claim 82, wherein the opportunistic microorganism is *Salmonella*, *Escherichia coli*, or *Campylobacter*.
85. A method for improving the growth performance of a subject comprising administering to the subject an effective amount the composition of any one of claims 1-68, the feed additive composition of any one of claims 69 or 70, or the feed of any one of claims 71-74, wherein improving the performance of a subject comprises of one or more of feed conversion ratio (FCR), weight gain, feed efficiency, carcass quality, reducing mortality, reducing morbidity, feed intake, average daily gain, carcass gain, bone mineralization, egg production, reduced digestive tract dysbiosis or dysfunction, milk composition, and/or milk production compared to the performance of a subject that has not been administered the feed additive composition or feed.
86. A method for increasing starch digestibility, lowering fecal starch output, and/or preventing a decrease in the pH in the lower gastrointestinal tract in a subject comprising adding an effective amount of the composition of any one of claims 1-68, the feed additive composition of any one of claims 69 or 70 to a feed for administration to a subject, wherein the subject exhibits one or more of increased starch digestibility and/or lowered fecal starch output compared to a subject that has not been administered the feed additive composition.
87. A method for increasing operational efficiency of a farm, comprising administering to a subject on the farm an effective amount of the composition of any one of claims 1-68, the feed additive composition of any one of claims 69 or 70, or the feed of any one of claims 71-74, wherein the increased operational efficiency results decreased labor costs, decreased roughage transport costs, and decreased amount of roughage added to a feed or feed additive.
88. The method of any one of claims 77-87, wherein the subject is a ruminant.

89. The method of claim 88, wherein the ruminant is selected from the group consisting of cattle, goats, sheep, giraffes, deer, gazelles, buffalo, reindeer, and antelopes.
90. The method of claim 89, wherein the cattle are beef cattle or dairy cattle.
91. The method of any one of claims 76-87, wherein the subject is a non-ruminant.
92. The method of claim 91, wherein the non-ruminant is selected from the group consisting of: equine, poultry, and swine.
93. The method of claim 92, wherein the poultry is selected from the group consisting of: a chicken, a goose, a duck, a quail, a turkey, broiler, broiler breeder, a layer, or a pigeon.
94. The method of claim 92, wherein the poultry is a chicken.
95. The method of any one of claims 77-94, wherein the composition of any one of claims 1-66, feed additive composition of claims 69 or 70, or feed of any one of claims 71-74 is provided to the subject for daily administration or for weekly administration.