sterility is required for inoculating each culture vessel, including the main production fermenter.

The present invention avoids the need for seed lines at the production site and provides simple means for sterile inoculation, thus saving both capital expenditure (CAPEX) and operational expenditure (OPEX). Compositions of dried or partially-dried spores as disclosed herein can be easily transported to organic waste management sites, stored and removed from storage upon need. It was surprisingly found that the spores in the dried or partially-dried compositions can successfully recover from storage, germinate and ferment organic waste to lactic acid at high yields. Advantageously, the dried or partially-dried compositions of spores do not require cooling and sustain various storage conditions for prolonged periods of time. As exemplified hereinbelow, viability of the spores is maintained throughout storage, and cell loss following drying and storage is minimal.

The utilization of organic waste as a substrate for fermentation as described herein is highly advantageous ompared to previously described lactic acid production processes which utilize source materials that are of high value as human food.

As further disclosed herein, the dried or semi-dried compositions of spores can be inoculated into the fermenter together with a saccharide-degrading enzyme to obtain simultaneous saccharification and fermentation. Remarkably, no or minimal lag time is observed until lactic acid is produced.

In one aspect, the present disclosure refers to a method for recycling organic waste to produce lactic acid or a salt thereof, the method comprising:

(i) providing a pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization, wherein the organic waste is food waste;

(ii) providing a dried or partially-dried composition of *Bacillus coagulans* spores, wherein the dried composition of *B. coagulans* spores is characterized by moisture content of less or equal to 10% w/w, and the partially-dried composition is characterized by a moisture content in the range of 15%-30% (w/w);

(iii) mixing the pretreated organic waste in a fermentation reactor with one or more saccharide-degrading enzyme and the dried composition of *B. coagulans* spores, and incubating the mixture in the fermentation reactor to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores, wherein an alkaline compound is added to the fermentation reactor during the incubating to adjust the pH in the range of 5 – 7; and

(iv) recovering lactic acid or a salt thereof from the fermentation broth.

According to one example, the present invention provides a method for

recycling organic waste to produce lactic acid or a salt thereof, the method comprising:

(i) providing a pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization;

(ii) providing a dried composition of Bacillus coagulans spores;

(iii) mixing the pretreated organic waste in a fermentation reactor with one or more saccharide-degrading enzyme and the dried composition of B. coagulans spores, and incubating the mixture in the fermentation reactor to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative B. coagulans cells that germinate from the spores; and

(iv) recovering lactic acid or a salt thereof from the fermentation broth.

In some embodiments, the method further comprises suspending the dried

composition of *B. coagulans* spores in a magnesium hydroxide slurry prior to the mixing with the pretreated organic waste in step (iii), thereby obtaining a *B. coagulans* spore suspension in which microbial contaminants are inactivated. In some embodiments, the concentration of magnesium hydroxide in the slurry is in the range of 1%-25%. In additional embodiments, the concentration of magnesium hydroxide in the slurry is in the range of 10%-20%. In yet additional embodiments, the concentration of magnesium hydroxide in the slurry is in the range of 5%-25%. Exemplary concentrations of magnesium hydroxide include 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%. Each possibility represents separate embodiment.

Suspension in magnesium hydroxide may be carried out for a few minutes up to several hours. Preferably, the suspending in a magnesium hydroxide slurry comprises incubating the suspension for between 15 minutes to 3 hours at a temperature between 25-60^{o}C, preferably at a temperature between 50-60^{o}C. In some embodiments, the suspending in a magnesium hydroxide slurry comprises incubating the suspension for 15-90min at a temperature between 25-60^{o}C. In additional embodiments, the suspending in a magnesium hydroxide slurry comprises incubating the suspension for 15-90min at a temperature between 50-55^{o}C. In some embodiments, the suspending in a magnesium hydroxide slurry comprises incubating the suspension for 30-90min or 30-60min at a temperature between 25-60^{o}C. Each possibility represents a separate embodiment. In additional embodiments, the suspending in a magnesium hydroxide slurry comprises incubating the suspension for 30-90min or 30-60min at a temperature between 50-55^{o}C. In some embodiments, suspension in a magnesium hydroxide slurry is carried out at room temperature.

In some embodiments, the dried composition of *B. coagulans* spores comprises magnesium lactate.

In some embodiments, the organic waste is selected from the group consisting of food waste, municipal waste, agricultural waste, plant material and a mixture or combination thereof.

In some embodiments, the incubating is carried out at a pH in the range of 5-7. In some particular embodiments, the incubating is carried out at a pH in the range of 5.5 – 6.5.

In some embodiments, the incubating is carried out at a temperature in the range of 45 – 60°C. In some particular embodiments, the incubating is carried out at a temperature in the range of 50-55°C.

In some embodiments, the incubating in step (iii) is carried out for a period of time in the range of 20-48 hours. In some particular embodiments, the incubating in step (iii) is carried out for a period of time in the range of 20-36 hours.

In some embodiments, the one or more saccharide-degrading enzyme is a polysaccharide-degrading enzyme selected from the group consisting of an amylase, a cellulase and a hemicellulose.

In some embodiments, the one or more saccharide-degrading enzyme comprises a glucoamylase.

In some embodiments, the mixing in step (iii) comprises adding the dried composition of *B. coagulans* to the fermentation reactor to obtain at least 10^4 spores/ml fermentation medium. In additional embodiments, the mixing in step (iii) comprises adding the dried composition of *B. coagulans* to the fermentation reactor to obtain at least 10^6 spores/ml fermentation medium.

A dried inoculum of spores as disclosed is characterized by moisture content of up to 15% (w/w) or any amount therebetween. In some embodiments, the dried composition of *B. coagulans* spores is characterized by moisture content of up to 10% (w/w). In some embodiments, the dried composition of *B. coagulans* spores is characterized by moisture content of 4%-15% (w/w), for example 4%-10% (w/w). Each possibility represents a separate embodiment of the present invention.

As provided herein, the moisture content of a dried or semi-dried inoculum, formulation or composition comprising *B. coagulans* spores refers to the amount of water outside the spores (namely, “moisture content” as used herein does not include water found inside the spores). The moisture content is provided as a percentage out of the total weight of the inoculum, formulation or composition. The terms “inoculum”, “formulation” and “composition” of spores are used herein interchangeably to describe a composition containing the spores, wherein the composition may be dried or semi-dried.

According to a further aspect, the present invention provides a system for recycling organic waste to produce lactic acid or a salt thereof, the system comprising:

(a) a source of pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization, wherein the organic waste is food waste;

(b) a dried composition of *Bacillus coagulans* spores;

(c) one or more saccharide-degrading enzyme; and

(d) a fermentation reactor for mixing therein the pretreated organic waste, the one or more saccharide-degrading enzyme and the dried composition of *B. coagulans* spores,

wherein the mixture is incubated in the fermentation reactor to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores, wherein an alkaline compound is added to the fermentation reactor during the incubating to adjust the pH in the range of 5 – 7.

In some embodiments, the system comprises:

(a) a source of pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization;

(b) a dried composition of *B. coagulans* spores suspended in a magnesium hydroxide slurry;

(c) one or more saccharide-degrading enzyme; and

(d) a fermentation reactor for mixing therein the pretreated organic waste, the one or more saccharide-degrading enzyme and the dried composition of *B. coagulans* spores suspended in a magnesium hydroxide slurry,

wherein the mixture is incubated in the fermentation reactor to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores.

According to a further aspect, the present invention provides a dried inoculum in a powder form for lactic acid fermentation, comprising spores of *Bacillus coagulans*; and magnesium lactate, wherein the inoculum is dried and ready for inoculation into a lactic acid production fermenter to provide lactic acid production.

In some embodiments, the dried inoculum comprises 10^8 – 10^10 spores/g powder, and the concentration of the magnesium lactate in the dried inoculum is in the range of 40-60% (w/w).

In some embodiments, a method for recycling organic waste to produce lactic acid or a salt thereof is provided, the method comprising:

(i) providing the dried inoculum comprising *B. coagulans* spores and magnesium

lactate;

(ii) suspending the dried inoculum in a magnesium hydroxide slurry, thereby obtaining a *B. coagulans* spore suspension in which microbial contaminants are inactivated;

(iii) mixing the suspension obtained in step (ii) in a fermentation reactor with one or more saccharide-degrading enzyme and pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization, and incubating to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores; and

iv) recovering lactic acid or a salt thereof from the fermentation broth.

The methods disclosed herein are particularly beneficial for the production of magnesium lactate. In some embodiments, the method is a method for producing magnesium lactate. In some embodiments, a method for recycling organic waste to produce magnesium lactate is provided, the method comprising:

providing a pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization;

providing a dried composition of *B. coagulans* spores, comprising *B. coagulans* and magnesium lactate;

suspending the dried composition of *B. coagulans* spores in a magnesium hydroxide slurry, thereby obtaining a *B. coagulans* spore suspension in which microbial contaminants are inactivated;

mixing the pretreated organic waste in a fermentation reactor with one or more saccharide-degrading enzyme and the *B. coagulans* spore suspension;

incubating the mixture in the fermentation reactor to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores, wherein an alkaline compound selected from magnesium hydroxide, magnesium oxide and magnesium carbonate is added to the fermentation reactor during the incubation to adjust pH, thereby obtaining lactate monomers and Mg^{2+} ions; and

recovering magnesium lactate from the fermentation broth.

In some particular embodiments, the alkaline compound added to the fermentation

reactor during the incubation to adjust pH is magnesium hydroxide.

According to a further aspect, there is provided a method for recycling organic waste to produce lactic acid or a salt thereof, the method comprising:

(i) providing the dried inoculum as described herein, comprising *B. coagulans* spores and magnesium lactate;

(ii) suspending the dried inoculum in a magnesium hydroxide slurry, thereby obtaining a *B. coagulans* spore suspension in which microbial contaminants are inactivated;

(iii) mixing the suspension obtained in step (ii) in a fermentation reactor with one or more saccharide-degrading enzyme and pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization, wherein the organic waste is food waste, and incubating to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores, wherein an alkaline compound is added to the fermentation reactor during the incubating to adjust the pH in the range of 5 – 7; and

(iv) recovering lactic acid or a salt thereof from the fermentation broth.

According to a further aspect, there is provided a method for recycling organic waste to produce lactic acid, the method comprising:

inoculating a dried or partially-dried composition of *Bacillus coagulans* spores into a lactic acid-production fermenter with pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization;

wherein the spores of *B. coagulans* from the dried or partially-dried inoculum successfully germinate in the fermenter and ferment the organic waste to produce lactic acid,

wherein the organic waste is food waste,

wherein the dried composition of *B. coagulans* spores is characterized by moisture content of less or equal to 10% w/w, and the partially-dried composition is characterized by a moisture content in the range of 15%-30% (w/w), and

wherein an alkaline compound is added to the fermentation reactor during the incubating to adjust the pH in the range of 5 – 7.

In some embodiments, the partially-dried composition of *B. coagulans* spores is characterized by a moisture content in the range of 15%-25% (w/w).

Other objects, features and advantages of the present invention will become clear from the following description and examples.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Inhibition of live microbial cells by $Mg(OH)_2$. *Escherichia coli* BL21, *Bacillus subtilis* strain 169 and *Saccharomyces cerevisiae* were incubated in LB **(A)** or 15% $Mg(OH)_2$ **(B)** at 52ºC for 2 hours, and subsequently plated on LB agar plates. Growth on the plates was examined following an overnight incubation at 52ºC.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to industrial fermentation processes for production of lactic acid from organic waste, in which a dried or semi-dried inoculum of *Bacillus coagulans* spores is used.

Organic waste management facilities handle collection, transport, processing, recycling/disposal and monitoring of waste materials. In order to recycle the waste into useful chemicals such as lactic acid, namely, utilize the organic waste as a substrate for industrial fermentation processes, an on-site fermentation system is typically required.

CLAIMS

1. A method for recycling organic waste to produce lactic acid or a salt thereof, the method comprising:

   (i) providing a pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization, wherein the organic waste is food waste;

   (ii) providing a dried or partially-dried composition of *Bacillus coagulans* spores, wherein the dried composition of *B. coagulans* spores is characterized by moisture content of less or equal to 10% w/w, and the partially-dried composition is characterized by a moisture content in the range of 15%-30% (w/w);

   (iii) mixing the pretreated organic waste in a fermentation reactor with one or more saccharide-degrading enzyme and the dried composition of *B. coagulans* spores, and incubating the mixture in the fermentation reactor to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores, wherein an alkaline compound is added to the fermentation reactor during the incubating to adjust the pH in the range of 5 – 7; and

   (iv) recovering lactic acid or a salt thereof from the fermentation broth.

2. The method of claim 1, further comprising suspending the dried composition of *B. coagulans* spores in a magnesium hydroxide slurry prior to the mixing with the pretreated organic waste in step (iii), thereby obtaining a *B. coagulans* spore suspension in which microbial contaminants are inactivated.

3. The method of claim 2, wherein the concentration of magnesium hydroxide in the slurry is in the range of 1%-25%.

4. The method of claim 2, wherein the suspending in a magnesium hydroxide slurry comprises incubating the suspension for 15-90min at a temperature between 25-60^{o}C.

5. The method of claim 1, wherein the dried composition of *B. coagulans* spores comprises magnesium lactate.

6. The method of claim 1, wherein the incubating in step (iii) is carried out at a temperature in the range of 45 – 60°C.

7. The method of claim 1, wherein the incubating in step (iii) is carried out for a period of time in the range of 20 – 48 hours.

8. The method of claim 1, wherein the one or more saccharide-degrading enzyme is a polysaccharide-degrading enzyme selected from the group consisting of an amylase, a cellulase and a hemicellulase.

9. The method of claim 1, wherein the one or more saccharide-degrading enzyme comprises a glucoamylase.

10. The method of claim 1, wherein the mixing in step (iii) comprises adding the dried composition of *B. coagulans* to the fermentation reactor to obtain at least 10^4 spores/ml fermentation medium.

11. A system for recycling organic waste to produce lactic acid or a salt thereof, the system comprising:

    (a) a source of pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization, wherein the organic waste is food waste;

    (b) a dried composition of *Bacillus coagulans* spores;

    (c) one or more saccharide-degrading enzyme; and

    (d) a fermentation reactor for mixing therein the pretreated organic waste, the one or more saccharide-degrading enzyme and the dried composition *of B. coagulans* spores,

    wherein the mixture is incubated in the fermentation reactor to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores, wherein an alkaline compound is added to the fermentation reactor during the incubating to adjust the pH in the range of 5 – 7.

12. The system of claim 11, wherein the system comprises:

    (a) a source of pretreated organic waste that was subjected to pretreatment

comprising reduction of particle size and optionally sterilization;

(b) a dried composition of *B. coagulans* spores suspended in a magnesium hydroxide slurry;

(c) one or more saccharide-degrading enzyme; and

(d) a fermentation reactor for mixing therein the pretreated organic waste, the one or more saccharide-degrading enzyme and the dried composition of *B. coagulans* spores suspended in a magnesium hydroxide slurry,

wherein the mixture is incubated in the fermentation reactor to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores.

13. A dried inoculum in a powder form for lactic acid fermentation, comprising spores of *Bacillus coagulans* and magnesium lactate, wherein the inoculum is dried and ready for inoculation into a lactic acid production fermenter to provide lactic acid production.

14. The dried inoculum of claim 13, wherein the dried inoculum comprises 10^8 – 10^10 spores/g powder, and wherein the concentration of the magnesium lactate in the dried inoculum is in the range of 40-60% (w/w).

15. A method for recycling organic waste to produce lactic acid or a salt thereof, the method comprising:

(i) providing the dried inoculum of claim 13, comprising *B. coagulans* spores and magnesium lactate;

(ii) suspending the dried inoculum in a magnesium hydroxide slurry, thereby obtaining a *B. coagulans* spore suspension in which microbial contaminants are inactivated;

(iii) mixing the suspension obtained in step (ii) in a fermentation reactor with one or more saccharide-degrading enzyme and pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization, wherein the organic waste is food waste, and incubating to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores, wherein an alkaline compound is added to the fermentation reactor during the incubating to

adjust the pH in the range of 5 – 7; and

(iv) recovering lactic acid or a salt thereof from the fermentation broth.

16. The method of claim 15, wherein the method is a method for producing magnesium lactate, comprising the following steps:

providing a pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization;

providing the dried inoculum of claim 13, comprising *B. coagulans* spores and magnesium lactate;

suspending the dried inoculum of *B. coagulans* spores in a magnesium hydroxide slurry, thereby obtaining a *B. coagulans* spore suspension in which microbial contaminants are inactivated;

mixing the pretreated organic waste in a fermentation reactor with one or more saccharide-degrading enzyme and the *B. coagulans* spore suspension;

incubating the mixture in the fermentation reactor to saccharify the organic waste and induce germination of the spores and subsequently lactic acid production by vegetative *B. coagulans* cells that germinate from the spores, wherein an alkaline compound selected from magnesium hydroxide, magnesium oxide and magnesium carbonate is added to the fermentation reactor during the incubation to adjust pH, thereby obtaining lactate monomers and Mg^{2+} ions; and

recovering magnesium lactate from the fermentation broth.

17. The method of claim 19, wherein the alkaline compound added to the fermentation reactor during the incubation to adjust pH is magnesium hydroxide.

18. A method for recycling organic waste to produce lactic acid, the method comprising:

inoculating a dried or partially-dried composition of *Bacillus coagulans* spores into a lactic acid-production fermenter with pretreated organic waste that was subjected to pretreatment comprising reduction of particle size and optionally sterilization;

wherein the spores of *B. coagulans* from the dried or partially-dried inoculum successfully germinate in the fermenter and ferment the organic waste to produce lactic acid,

wherein the organic waste is food waste,

wherein the dried composition of *B. coagulans* spores is characterized by moisture content of less or equal to 10% w/w, and the partially-dried composition is characterized by a moisture content in the range of 15%-30% (w/w), and

wherein an alkaline compound is added to the fermentation reactor during the incubating to adjust the pH in the range of 5 – 7.