Microbial Fermentation

Field of the Invention

The present invention relates to a method of controlling a microbial fermentation of a microorganism capable of converting one or more gases into one or more organic acids in a fermentation broth. Such a microbial fermentation also forms part of the present invention.

Background

Many chemicals require fossil fuels for their production. At the same time, many industrial processes produce carbon dioxide (CO_2), and solutions for effective carbon capture from such processes are being sought. Acetogenic bacteria are a group of bacteria capable of utilising CO_2 (or carbon monoxide [CO]) as the sole carbon source, or a combination of CO_2 and CO as carbon sources. The use of acetogenic bacteria has therefore been proposed for capturing CO_2 from industrial processes. At the same time, these bacteria can also be used to produce useful chemicals, such as acetone.

Often, maintaining sterile conditions in microbial fermentations (such as acetogenic fermentations) is difficult, and so non-sterile conditions results. Typically, the growth of contaminating microorganisms during fermentation will diminish fermentation productivity and so the process must be stopped and restarted after cleaning and sterilization of the fermentation vessel. The requirement to maintain low amounts of contaminating microorganisms in fermentation processes often prevents long-term, stable, continuous operation and result in the need for costly and time-consuming sterilization. Common contaminating microorganisms in acetogenic bacteria fermentations include methanogens.

Zhang *et al., 2018* identified that free acetic acid concentration in a mesophilic mixed culture fermentation broth was correlated with methanogenic activity in such a fermentation. The pH of the fermentation broth was controlled in order to provide the desired free acetic acid concentration (with lower pH values providing higher free acetic acid concentrations).

Diender *et al., 2023* identified that acetogenic carboxydotrophs are able to convert CO into ethanol under certain conditions. In particular, Diender *et al.* used a mesophilic fermentation of *Clostridium autoethanogenum* and demonstrated that increased alcohol production by *Clostridium authoethanogenum* can be triggered by operating the fermentation at lower pH values.

EP2268824A1 relates to a method for sustaining a mesophilic acetogen microorganism fermentation of syngas for the production of ethanol in the event of limited or cut syngas supply to the fermentation. The authors suggest sustaining the fermentation by supplying CO_2 to allow the microorganisms to temporarily metabolise ethanol back to acetate. In order to avoid the free acetic acid concentration in the fermentation broth rising above 5 g/L, at which point microorganisms in the fermentation are inhibited, EP2268824A1 suggests raising the pH of the fermentation broth whilst supplying CO_2 in order to shift free acetic acid

back into its dissociated form. EP2268824A1 uses mesophilic bacterium *Clostridium Ljungdahlii* for the production of ethanol in its fermentation.

However, there remains a need in the art for improved bioprocesses for capturing CO_2 and/or CO from industrial processes.

Summary of the Invention

It is desired to provide a method of controlling a microbial fermentation of a thermophilic microorganism capable of converting one or more gases into one or more organic acids such that: a) growth of contaminant microorganisms, in particular, but not exclusively, methanogens, in the fermentation broth is reduced; and b) a high yield based on substrate of the one or more organic acids is provided.

Accordingly, in a first aspect there is provided a method of controlling a microbial fermentation of a microorganism in a fermentation broth, said microorganism being capable of converting one or more gases into one or more organic acids, wherein: said microorganism is thermophilic; and the method comprises: controlling (e.g. varying) the pH of the fermentation broth such as to provide a free acid concentration in the fermentation broth of greater than 8 mM; and maintaining the alcohol concentration in the fermentation broth at less than 100 mM throughout the fermentation. Use of the method according to the first aspect to control such a fermentation is expected to result in the fermentation simultaneously having a high yield based on substrate (by limiting alcohol by-product production) and low contaminant microorganism growth (by inhibiting said contaminant microorganisms using a high free acid concentration).

Free acid may be defined as undissociated acid (i.e. in its protonated form). By way of example, undissociated acetic acid is CH_3COOH. The free acid concentration in the fermentation broth may be defined as the sum of the concentrations of one or more acids in their protonated form (i.e. the sum of individual free acid concentrations) in the supernatant of the fermentation broth. The free acid concentration may be the free acid concentration of the one or more organic acids (for example, if no inorganic acid species were present in the fermentation broth).

The fermentation may have said one or more organic acids as the target product. Conveniently, the target product of the fermentation is thus also the source of the compound for inhibiting contaminant microorganisms within the fermentation. The method may comprise maintaining a total acid concentration of said one or more acids in the fermentation broth of greater than 80 mM. The method may comprise maintaining the total acid concentration of said one or more acids (e.g. one or more organic acids) in the fermentation broth at greater than 0.15 M, 0.25 M, 0.35 M, 0.45 M, 0.55 M, 0.65 M, 0.75 M, 0.85 M, 0.95 M, 1.05 M, or 1.15 M. Maintaining a high concentration of said one or more acids can avoid the need to maintain the fermentation broth at a very low pH (e.g. less than 2) in order to provide the free acid concentration in the fermentation broth of greater than 80 mM. Moreover, with organic acid as the target product of the fermentation, efficient harvesting of the product may be facilitated by maintaining a high concentration of said product in the fermentation broth. The total acid concentration of said one or more acids in the fermentation broth may be defined as the sum of the concentrations of the free acid and

dissociated/ionised acid forms of the one or more acids. Similarly, the total acid concentration of said one or more organic acids in the fermentation broth may be defined as the sum of the concentrations of the free acid and dissociated/ionised acid forms of the one or more organic acids.

The method may comprise maintaining the total acid concentration of said one or more acids (e.g. the one or more organic acids) at less than 1.15 M, 1.05M. 0.95 M, 0.85 M, 0.75 M, 0.65M or 0.55 M.

The method may comprise maintaining the total acid concentration of said one or more acids at greater than 0.15 M and less than 1.15 M, for example, at greater than 0.35 M and less than 1.05 M.

The method may further comprise maintaining the alcohol concentration in the fermentation broth at less than 50 mM, less than 20 mM, less than 10 mM, or less than 2 mM. Advantageously, maintaining a low alcohol concentration in the fermentation broth increases the yield based on substrate of the one or more organic acids and limits inhibition of the thermophilic microorganisms by the one or more alcohols. Maintaining the alcohol concentration in the fermentation broth at less than a given value also encompass there being no, or trace amounts of, alcohol present in the fermentation broth. The step of maintaining the alcohol concentration in the fermentation broth below a given value may be conducted by selection of the microorganism for the microbial fermentation (i.e. it may not involve a control action during the fermentation), however, the alcohol concentration may also be actively controlled during the fermentation, for example by modification of the fermentation pH and/or redox potential (e.g. to select which metabolism pathway(s) are active for the microorganism).

The ratio of the alcohol concentration in the fermentation broth to the free acid concentration in the fermentation broth may be less than or equal to 2:1, e.g. less than or equal to 1:1, less than or equal to 2:3, or less than or equal to 1:2, or less than or equal to 1:3. Most preferably, the ratio of the alcohol concentration in the fermentation broth to the free acid concentration in the fermentation broth may be less than or equal to 1:3.

The ratio of the alcohol concentration in the fermentation broth to the total organic acid concentration in the fermentation broth may be less than or equal to 1:10, e.g. less than or equal to 1:15, less than or equal to 1:20, or less than or equal to 1:40. Most preferably, the ratio of the alcohol concentration in the fermentation broth to the free acid concentration in the fermentation broth may be less than or equal to 1:20.

The one or more gasses may be provided to the fermentation as a substrate, e.g. the thermophilic microorganism may grow on said one or more gases as the main carbon source. The one or more gases may comprise CO_2, CO or a mixture thereof. The one or more gases may further comprise hydrogen (H_2). The one or more gases may by syngas (i.e. a mixture comprising CO and H_2, and optionally further comprising CO_2, methane (CH_4) and/or trace gases. In this way, the fermentation can produce organic acids from byproduct gases from industrial processes and/or from gasification of biomass.

In the present application, a microorganism being thermophilic may be defined as the microorganism being capable of growing at a temperature above 45°C.

The method may further comprise maintaining the temperature of the fermentation broth above 45°C. Operating at above 45°C can increase the productivity of the organic acid-producing thermophilic microorganism whilst inhibiting the growth of mesophilic contaminant microorganisms. The method may comprise maintaining the temperature of the fermentation broth above 50°C, 55°C, 60°C, or 65°C. The method may comprise maintaining the temperature of the fermentation broth below 75°C, 70°C, 65°C, 60°C or 55°C. The upper bound of the temperature of the fermentation broth may be set based on the temperature that the organic acid-producing thermophilic microorganism can withstand without being inhibited. The method may comprise maintaining the temperature above 45°C and below 75°C, for example, above 55°C and below 65°C.

The method may comprise controlling (e.g. varying) the pH of the fermentation broth such as to provide a free acid concentration in the fermentation broth of greater than or equal to 10 mM, greater than or equal to 15 mM, greater than or equal to 20 mM, greater than or equal to 25 mM, greater than or equal to 30 mM, greater than or equal to 35 mM, greater than or equal to 40 mM, greater than or equal to 45 mM, greater than or equal to 50 mM, greater than or equal to 55 mM, greater than or equal to 60 mM, greater than or equal to 65 mM, greater than or equal to 70 mM, greater than or equal to 75 mM, greater than or equal to 80 mM, or greater than or equal to 85 mM. It can be appreciated that modifying organic-acid producing microorganism (e.g. *Moorella, such as Moorella thermoaceticum*, *Thermoanaerobacter*, *Thermoanaerobacterium*, *Acetogenum*, *Carboxydothermus*, or *Acetobaterium)* can provide such a microorganism with a higher tolerance for free acid concentrations in the fermentation broth. Such a modified microorganism can be used in a process where the pH is controlled such as to provide a free acid concentration of greater than or equal to 90 mM, greater than or equal to 100 mM, greater than or equal to 110 mM, greater than or equal to 115 mM, greater than or equal to 150 mM, greater than or equal to 200 mM, greater than or equal to 250 mM, or greater than or equal to 300 mM. In this way, the growth and activity of contaminant microorganisms (e.g. methanogenic bacteria) in the fermentation broth can be inhibited. Controlling (e.g. varying) the pH of the fermentation broth to provide such free acid concentrations in the fermentation broth can provide a relative abundance of the organic acid-producing thermophilic microorganism(s) in the fermentation broth with respect to all other non-organic acid-producing microorganisms in the fermentation broth that is greater than or equal to 0.25, greater than or equal to 0.4, greater than or equal to 0.5, or greater than or equal to 0.6. Operating the fermentation such that the free acid concentration is substantially above the concentration at which the contaminant microorganisms are inhibited can be advantageous for controlling the growth of the organic acid-producing microorganism and also facilitating operation of the fermentation process at the desired pH even as total acid concentration of said one or more acids in the fermentation broth continues to increase due to growth and activity of the organic acid-producing microorganism.

The method may comprise controlling (e.g. varying) the pH of the fermentation broth such as to provide a free acid concentration in the fermentation broth of less than or equal to 300 mM, less than or equal to 250 mM, less than or equal to 200 mM, less than or equal to 150 mM, less than or equal to 115 mM less than or equal to 110 mM, less than or equal to 100 mM, less than or equal to 90 mM, less than or equal to 80 mM, less than or equal to 70 mM, less than or equal to 65 mM, less than or equal to 60 mM, less than

or equal to 50 mM, less than or equal to 40 mM, less than or equal to 35 mM, less than or equal to 30 mM, less than or equal to 25 mM, less than or equal to 20 mM, or less than or equal to 15 mM. Accordingly, the free acid concentration can be set such that the organic acid-producing microorganism is not inhibited, whilst still inhibiting contaminant microorganisms.

The method may comprise controlling (e.g. varying) the pH of the fermentation broth such as to provide a free acid concentration in the fermentation broth of greater than 8 mM and less than 300 mM, for example, greater than or equal to 20 mM and less than or equal to 90 mM, e.g. greater than or equal to 30 mM and less than or equal to 75 mM.

It can be appreciated that the concentrations of the products and by-products resulting from the microbial fermentation are typically a product of both the microorganism being grown and the conditions under which it is growing. The person skilled in the art understands how to vary the conditions of the microbial fermentation and can observe the effect of these variations on the concentrations of the products and by-products. Accordingly, with the knowledge provided by the present application of the beneficial concentrations of the products and by-products in the fermentation and the preferred microorganisms, the skilled person would be able to vary the conditions of the microbial fermentation to achieve the concentrations of the products and by-products recited herein without undue burden.

The method may comprise maintaining the free acid concentration in the fermentation broth above 8 mM by varying the pH of the fermentation broth based on the free acid concentration and/or the total acid concentration, and the pKa of the one or more organic acids.

The step of varying the pH of the fermentation broth may comprise reducing the pH of the fermentation broth in response to a reduction in the total acid concentration of the one or more organic acids in the fermentation broth.

The step of varying the pH of the fermentation broth may comprise increasing the pH of the fermentation broth in response to an increase in the total acid concentration of the one or more organic acids in the fermentation broth.

In some instances, where the growth rates of the microorganism and organic acid production rates of the microorganism are understood, the pH may be varied without measuring the total acid concentration of the one or more organic acids in the fermentation broth. For example, if it were known that during fermentation of a microorganism, the total acid concentration of the one or more organic acids in the fermentation broth increases at a set point in time after fermentation commenced, the pH could be increased to maintain the free acid concentration at a set point free acid concentration (e.g. above 8 mM) without measuring/detecting the increase in the total acid concentration of the one or more organic acids at that point in time.

The thermophilic microorganism may be selected from the group consisting of: thermophilic acetogens; thermophilic propionibacteria; thermophilic butyric acid bacteria; thermophilic lactic acid bacteria; thermophilic succinic acid bacteria; and hydrogen oxidising bacteria; thermophilic pyruvic acid bacteria; thermophilic tartaric acid bacteria; thermophilic iso-butyric acid bacteria; thermophilic hydroxybutyric acid bacteria; and thermophilic hydroxyvaleric acid bacteria. Preferably, the thermophilic microorganism may

be selected from the group consisting of: thermophilic acetogens; thermophilic propionibacteria; thermophilic butyric acid bacteria; thermophilic lactic acid bacteria; thermophilic succinic acid bacteria; and hydrogen oxidising bacteria. Fermentation of such bacteria can produce commonly used chemical feedstocks. An acetogen may be defined as a microorganism having a metabolism converting CO_2 (with H_2) or CO into acetyl-coenzyme-A. A propionibacterium may be defined as a microorganism having a metabolism converting CO_2 (with H_2) or CO into propionyl-coenzyme-A. A butyric acid bacterium may be defined as a microorganism having a metabolism converting CO_2 (with H_2) or CO into butyryl-coenzyme-A. A lactic acid bacterium may be defined as a microorganism having a metabolism converting CO_2 (with H_2) or CO into lactyl-coenzyme-A. A succinic acid bacterium may be defined as a microorganism having a metabolism converting CO_2 (with H_2) or CO into succinyl-coenzyme-A. A hydrogen-oxidising bacterium may be defined as a microorganism having a metabolism capable of using hydrogen as an electron donor. A pyruvic acid bacterium may be defined as a microorganism having a metabolism converting CO_2 (with H_2) or CO into pyruvyl-coenzyme-A. A tartaric acid bacterium may be defined as a microorganism having a metabolism converting CO_2 (with H_2) or CO into tartryl-coenzyme-A. An iso-butyric acid bacterium may be defined as a microorganism having a metabolism converting CO_2 (with H_2) or CO into isobutyryl-coenzyme-A. A hydroxybutyric acid bacterium may be defined as a microorganism having a metabolism converting CO_2 (with H_2) or CO into hydroxybutyryl-coenzyme-A. A hydroxyvaleric acid bacterium may be defined as a microorganism having a metabolism converting CO_2 (with H_2) or CO into hydroxyvaleryl-coenzyme-A.

The microorganism may be selected from the group consisting of: *Moorella*, *Thermoanaerobacter*, *Thermoanaerobacterium*, *Acetogenum*, *Carboxydothermus*, and *Acetobaterium*. Preferably, the microorganism is *Moorella thermoaceticum*. Such microorganisms can provide high organic acid productivity with low productivity of the corresponding alcohol, even at low pH values (e.g. less than 7). The microorganism may comprise genes encoding alcohol dehydrogenase (AdhE) or aldehyde oxidoreductase (AOR). The microorganism may comprise genes encoding for specific AdhE and AOR enzymes corresponding to the organic acid(s) produced by that microorganism (e.g. acetaldehyde oxidoreductase for an acetic acid-producing microorganism; however, it is expected that many oxidoreductases and dehydrogenases are promiscuous with respect to the reaction they are catalysing, e.g. formaldehyde oxidoreductase reducing short-chain aldehydes other than formaldehyde (e.g. acetaldehyde). The present inventors have established that, even where the microorganism comprises genes encoding AdhE and AOR and thus produces said enzymes, which would typically catalyse the conversion of organic acids to alcohols in the fermentation broth at low pH, they do not do so under the above-described conditions (e.g. thermophilic fermentation, pH less than or equal to 7). The thermophilic microorganism may not be a member of the *Clostridium* genus.

The total organic acid productivity of the microbial fermentation may be greater than or equal to 8 mM/hour, greater than or equal to 12 mM/hour, greater than or equal to 17 mM/hour, greater than or equal to 25 mM/hour, greater than or equal to 33 mM/hour, greater than or equal to 40 mM /hour, or greater than or equal to 50 mM/hour. The total organic acid productivity may be calculated based on the organic acid produced by the microbial fermentation over a time period of 1 hour, 6 hours, 12 hours, 1

day, 2 days or 1 week. The total organic acid productivity may be defined as the productivity of both the free acid and dissociated/ionised acid forms.

Said one or more organic acids may be selected from the group consisting of acetic acid, butyric acid; propionic acid; lactic acid; succinic acid; pyruvic acid; tartaric acid; iso-butyric acid; hydroxybutyric acid; and hydroxyvaleric acid. Preferably, said one or more organic acids may be selected from the group consisting of acetic acid, butyric acid; propionic acid; lactic acid; and succinic acid. Most preferably, said one or more organic acids consists of acetic acid.

Where the fermentation is producing one or more organic acids, one or more alcohols may be produced in small amounts as a by-product. Said one or more alcohols may be selected from the group consisting of ethanol; butanol; propanol; propylene glycol; butane-1,4-diol; butane-1,2,3,4-tetrol; isobutanol; butane-1,3-diol; butane-1,2-diol; and pentane-1,4-diol. Preferably, said one or more alcohols may be selected from the group consisting of ethanol, butanol, propanol, propylene glycol and butane-1,4-diol. Most preferably, said one or more alcohols consists of ethanol.

The one or more organic acids may consist of propionic acid and the one or more alcohols may consist of propanol. The one or more organic acids may consist of butyric acid and the one or more alcohols may consist of butanol. The one or more organic acids may consist of lactic acid and the one or more alcohols may consist of propylene glycol. The one or more organic acids may consist of succinic acid and the one or more alcohols may consist of butane-1,4-diol.

The total alcohol productivity of the microbial fermentation may be less than or equal to 5 mM/hour. Preferably, the total alcohol productivity of the microbial fermentation may be less than or equal to 3 mM/hour, less than or equal to 2 mM/hour, less than or equal to 1 mM/hour, less than or equal to 0.5 mM/hour, less than or equal to 0.25 mM/hour, or less than or equal to 0.125 mM/hour. More preferably, no alcohol may be produced by the microbial fermentation (i.e. the total alcohol productivity may be zero). Such an alcohol productivity is advantageous where the target fermentation product is said one or more organic acids, as lower alcohol productivity provides higher yield based on substrate for the one or more organic acids and limits inhibition of the thermophilic microorganisms by the one or more alcohols.

The total alcohol productivity may be calculated based on the alcohol produced by the microbial fermentation over a time period of 1 hour, 6 hours, 12 hours, 1 day, 2 days or 1 week.

The microbial fermentation may be an industrial scale process. By way of example the fermentation may be at at least 1 m^3 scale, at least 5 m^3 scale, at least 50 m^3 scale, at least 150 m^3 scale, or at least 250 m^3 scale. For example, the fermentation broth may have a volume of at least 1 m^3, at least 5 m^3, at least 50 m^3, at least 150 m^3, or at least 250 m^3. The fermentation broth may have a volume of less than 500 m3 scale, less than 250 m^3, less than 150 m^3, less than 50 m^3, or less than 5 m^3. The fermentation broth may have a volume between 1 m^3 and 250 m^3, for example between 5 m^3 and 150 m^3, e.g. between 50 m^3 and 100 m^3.

The method may comprise conducting the step of controlling (e.g. varying) the pH of the fermentation broth during a period of continual supply of the one or more gases.

The method may comprise maintaining the fermentation broth at a pH less than 7. The pH can be used to adjust the equilibrium ratio of free acid to dissociated acid in the fermentation broth, and accordingly provide a set point (desired) free acid concentration to inhibit contaminant microorganisms. The pH to provide the set point (desired) free acid concentration is a function of the total acid concentration of the one or more acids in the fermentation broth. The set point (desired) free acid concentration in the fermentation broth may be greater than the free acid concentration required to inhibit contaminant microorganisms (e.g. greater than 8 mM). Maintaining the fermentation broth at a pH less than 7 can provide the set point (desired) free acid concentration with a typical total acid concentration in the fermentation broth. The method may comprise maintaining the fermentation broth at a pH less than 6, less than 5.2, less than 5 or less than 4. The method may comprise maintaining the fermentation broth at a pH greater than 4, greater than 4.5, greater than 5, greater than 5.2 or greater than 6. The method may comprise maintaining the fermentation broth at a pH less than 7 and greater than 4, for example, maintaining the fermentation broth at a pH less than 6 and greater than 4.5, or maintaining the fermentation broth at a pH less than 5.2 and greater than 4.5, e.g. less than 5 and greater than 4.5.

The method may comprise determining the free acid concentration or total acid concentration of the one or more organic acids (i.e. in the fermentation broth); determining a target pH of the fermentation broth; and controlling (e.g. varying) the pH of the fermentation broth to (substantially) maintain, reach (e.g. arrive at, achieve) and/or approach the target pH. In this way, the pH of the fermentation broth can be used as the control variable for providing the set point (desired) free acid concentration. Reaching/arriving at/achieving the target pH may be understood to include varying the pH with the aim of reaching/arriving at/achieving the target pH. Additionally, the pH may then increase above or fall below (depending on the direction from which the pH has approached the target pH) the target pH (e.g. the target pH may be reached/arrived at/achieved only instantaneously, or for a longer period of time). The skilled person would understand that in a real process, the pH may oscillate about the target pH due to an imperfect (i.e. real) control system and/or disturbances to the process. Accordingly, the target pH may be reached/arrived at/achieved only for a certain time period before the pH then deviates from the target pH.

The step of determining the target pH may be based on the total and/or free acid concentration in the fermentation broth, e.g. made by determining (e.g. calculating) the target pH that is needed to maintain the free acid concentration above 8 mM (or another lower bound on the free acid concentration) or needed to maintain the set point free acid concentration.

It can be understood that the method may comprise changing/varying the target pH during the course of the fermentation. This is in contrast to typical fermentations, where the target pH is typically constant throughout the fermentation. The target pH may change during the course of the fermentation such that, at a point in time (e.g. at multiple points in time, or at all points in time) in the fermentation, the free acid concentration in the fermentation broth at the target pH is greater than 8 mM. By way of example, at a first point in time during the course of the fermentation, a first target pH may be determined where there is a first concentration of one or more organic acids in the fermentation broth, and at a second point in time during the course of the fermentation, a second target pH may be determined where there is a second concentration of one or more organic acids in the fermentation broth, where the first point in time is

different to the second point in time, the first concentration is different to the second concentration, and thus the first target pH is different to the second target pH. Accordingly, the method may then comprise varying the pH of the fermentation broth to reach/achieve/arrive at/approach the (varying) target pH.

The step of determining the target pH may be made by determining (e.g. calculating) the target pH that is needed for the free acid concentration to reach the set point free acid concentration. The method may comprise determining the difference between the free acid concentration and the set point free acid concentration and determining the target pH based on said difference. The set point free acid concentration may be greater than or equal to the lower bound on the free acid concentration (e.g. greater than 8 mM). Where it is determined that the free acid concentration exceeds the set point free acid concentration, the method may comprise increasing the target pH (e.g. setting the target pH above the current pH of the fermentation broth). Where it is determined that the free acid concentration is less than the set point free acid concentration, the method may comprise reducing the target pH (e.g. setting the target pH below the current pH). The set point free acid concentration may be greater than 8 mM and less than 300 mM, for example, greater than or equal to 20 mM and less than or equal to 90 mM, e.g. greater than or equal to 30 mM and less than or equal to 75 mM.

The set point free acid concentration may be constant during the fermentation.

The target pH may be calculated based on the pKa of the one or more organic acids in the fermentation broth, the total acid concentration of each of said one or more acids in the fermentation broth, and the set point free acid concentration (e.g. the lower bound on the free acid concentration).

The method may comprise determining (e.g. calculating) a required change in the pH based on the free acid concentration and/or the total acid concentration, the pKa of the one or more organic acids, and the current pH. By way of example, the method may comprise calculating the target pH and determining the required change in the pH to reach the target pH (i.e. based on the current pH and the target pH. The method may further comprise determining the quantity of exogenous (inorganic) base or exogenous (inorganic) acid to be added to the fermentation broth to achieve the required change in the pH and/or determining a required change in a flowrate of exogenous (inorganic) base or exogenous (inorganic) acid into the fermentation broth to achieve the required change in the pH within a given time period.

In some instances, where the growth rates of the microorganism and organic acid production rates of the microorganism are understood, the target pH may be varied without measuring the total acid concentration of the one or more organic acids in the fermentation broth. For example, if it were known that during fermentation of a microorganism, the total acid concentration of the one or more organic acids in the fermentation broth increases at a set point in time after fermentation commenced, the target pH could be increased to maintain the free acid concentration at a set point free acid concentration without measuring/detecting the increase in the total acid concentration of the one or more organic acids at that point in time.

Determining the free acid concentration may comprise determining the total acid concentration; determining the pH of the fermentation broth; and calculating the free acid concentration of said one or more acids based on said total acid concentration and the pH of the fermentation broth. Determining the

total acid concentration of said one or more acids may comprise obtaining a sample of the fermentation broth, measuring the total acid concentration of the sample (e.g. using high-performance liquid chromatography (HPLC)). The total acid concentration of the sample may be taken as the total acid concentration of the fermentation. Determining the pH of the fermentation broth may comprise measuring the pH of the sample or measuring the fermentation broth inline.

The step of controlling (e.g. varying) the pH of the fermentation broth may comprise increasing the pH of the fermentation broth (e.g. upwards towards the target pH) to reduce the free acid concentration towards the desired free acid concentration. The step of controlling (e.g. varying) the pH of the fermentation broth may comprise decreasing the pH of the fermentation broth (e.g. downwards towards the target pH) to reduce the free acid concentration towards the desired free acid concentration. The pH of the fermentation broth may be increased by addition of a strong exogenous base to the fermentation broth. The pH of the fermentation broth may be reduced, for example, by allowing the fermentation to continue producing the one or more organic acids, and/or by addition of an exogenous acid to the fermentation broth. The step of controlling (e.g. varying) the pH of the fermentation broth may be carried out by an electronic control instrument e.g. a PID controller.

The microbial fermentation may be a batch fermentation or a batch-fed fermentation. However, preferably, the microbial fermentation is a continuous fermentation. Operation as a continuous fermentation is facilitated by the maintenance of the free acid concentration above 8 mM, such that contaminant microorganisms are inhibited.

In a second aspect there is provided a microbial fermentation of a microorganism in a fermentation broth, said microorganism being capable of converting one or more gases into one or more organic acids, wherein: said microorganism is thermophilic; the alcohol concentration in the fermentation broth is less than 100 mM; and a free acid concentration in the fermentation broth of greater than or equal to 8 mM.

Any one or more of the optional features set out in relation to the first aspect may be incorporated into the second aspect, except where such a combination is clearly impermissible or expressly avoided.

The concentrations in the summary above and in the detailed description and examples below are in units of molar concentration (M) (i.e. moles per dm^3). The concentration values can be converted to g/L by multiplying the mola concentration by the (average) molecular weight of the species concerned.

Summary of the Figures

Figure 1 provides a flow chart of an embodiment of the control method according to the first aspect;

Figure 2 illustrates the variation in concentration of free acetic acid with respect to time in a fermentation of a *Moorella* microorganism producing acetic-acid on a substrate of H_2 and CO_2, and the corresponding variation in the percentage of methane in the off-gas from the fermentation;

Figure 3 illustrates the relative abundance of the *Moorella* microorganism with respect to *Methanothermobacteria* and other contaminant strains within the fermentation at different times during the fermentation, the data in Figure 2 being obtained from the same fermentation as that in Figure 2; and

Figure 4 illustrates the trend between the concentration of free acetic acid in the fermentation broth and the percentage of methane in the off-gas from the fermentation.

Detailed Description of the Invention

Aspects and embodiments of the present disclosure will now be discussed with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

The present disclosure relates to a fermentation process and a method of controlling that fermentation. Whilst the following description and examples relate to a thermophilic (i.e. high temperature) fermentation of *Moorella* for the production of acetic acid, and with the aim of controlling the activity of contaminant microorganisms, in particular methanogens, the principles and features of the fermentation process and control method are equally applicable to other fermentation processes of thermophilic microorganisms capable of converting one or more gases into one or more organic acids. In particular, the disclosed processes and methods are particularly advantageous when applied to biotechnological processes using non-sterile features, like non-sterile reactors, non-sterile storage or handling methods, or non-sterile inputs or ingredients, and indeed biotechnological processes intended to be sterile, but which have become inadvertently contaminated.

Free organic acids (e.g. free acetic acid, CH_3COOH) are able to diffuse freely across the cell membranes of microorganisms. Once within the cell of the microorganism, the free organic acid may dissociate, thereby decreasing the intracellular pH of the microorganism. A sufficiently low intracellular pH results in deactivation of enzymes and other cellular machinery, causing inhibition of the microorganism. Different microorganisms have different intracellular pH values which they are able to tolerate before they become inhibited. In particular, microorganisms capable of converting one or more gases (e.g. a syngas mixture) into one or more organic acids are typically able to tolerate lower intracellular pH values than other microorganisms (e.g. methanogens).

This difference in intracellular pH tolerance between organic acid-producing microorganisms and other microorganisms can be utilised in fermentation processes seeking to produce organic acids as the target product of the fermentation process in order to selectively inhibit contaminant microorganisms.

By way of example, in a thermophilic gas fermentation generating acetic acid as the target product, the intended biocatalyst is an acetogen, a bacterium that naturally produces acetic acid as part of its core metabolism of gases such as CO or CO_2 (in the presence of H_2). As such, those acetogens have unique molecular mechanisms that allow them to naturally tolerate (i.e. maintaining activity and growth) free acetic acid concentrations within a range that cannot be tolerated by many other non-organic acid-producing microorganisms (for example, 15-30 mM free acetic acid), for example, contaminating bacteria

such as methanogenic archaea and thermophilic methanogenic bacteria. Such contaminating archaea and bacteria lack said unique molecular mechanisms for tolerating free acetic acid at concentrations in the fermentation broth above around 8 mM, and therefore are inhibited from generating undesirable by-products, such as methane, at such free acetic acid concentrations.

Acid species in the fermentation broth exist in both their free acid (i.e. protonated) form and their conjugate base and hydrogen ion pair form; these forms of the acid species form an equilibrium with an equilibrium constant, K_a. Taking the example of acetic acid, the equilibrium constant is defined by Equation 1 and thus, the concentration of free acetic acid within the fermentation broth is provided as in Equation 2, where $pK_a = -\log K_a$, $\mathrm{pH} = -\log[\mathrm{H}^+]$, and $[(\mathrm{CH_3COOH})_T]$ is the total acid concentration of acetic acid in the fermentation broth (i.e. including both the free acetic acid and dissociated/ionised acetic acid), which can be measured, for example, using HPLC.

Equation 1

$$K_a = \frac{[\mathrm{CH_3COO^-}][\mathrm{H^+}]}{[\mathrm{CH_3COOH}]}$$

Equation 2

$$[\mathrm{CH_3COOH}] = [(\mathrm{CH_3COOH})_T]\left(\frac{1}{10^{(pH-pK_a)}+1}\right)$$

Thus, the pH of the fermentation broth can be used to control the concentration of free acetic acid within the fermentation. The pH can be varied either using the organic acid productivity of the organic acid-producing microorganism(s) in the fermentation and/or by addition of exogenous acid or base into the fermentation broth.

As discussed above in relation to *Diender et al., 2023*, it is the expectation that in a fermentation process producing large quantities of acetic acid (e.g. having an acetic acid concentration greater than 80 mM) and operating at low pH (e.g. below pH 6) at least some of the acetic acid would be converted into ethanol. In particular, thermophilic microorganisms such as *Moorella*, *Moorella thermoaceticum*, *Thermoanaerobacter*, *Thermoanaerobacterium*, *Acetogenum*, *Carboxydothermus*, and *Acetobaterium* include genes encoding alcohol dehydrogenase (AdhE) and/or acetaldehyde oxidoreductase (AOR). As discussed in *Diender et al., 2023,* these enzymes form part of the metabolic pathways for conversion of organic acids, in particular acetic acid, into ethanol. However, as discussed below in relation to the examples, in a thermophilic fermentation of *Moorella thermoacetica* it was found that, despite encoding for said enzymes, ethanol production in more than trace amounts did not result, even with total acetic acid concentrations reaching in excess of 100 mM and a fermentation broth below pH 6.

Consequently, use of pH to control free acetic acid concentration, and accordingly the growth and activity of contaminant strains within a fermentation process of thermophilic organic acid-producing bacteria is viable. This is the case even where the target product is said organic acids and it is desired to maintain no alcohol or low alcohol concentrations within the fermentation broth in order to provide high yield based on substrate of said organic acids.

Accordingly, where a fermentation process of a thermophilic microorganism that is capable of converting one or more gases into one or more organic acids is provided, such as that described above and/or that described below in the examples, a method of controlling such a fermentation to constrain growth of contaminant microorganisms and production of by-products (e.g. methane and the corresponding alcohol(s) to said organic acid(s)) according to the first aspect described above can be used. Figure 1 provides a flow chart of an embodiment of the control method according to the first aspect.

An embodiment of the control method according to the first aspect comprises: controlling (e.g. varying) the pH of the fermentation broth such as to provide a free acid concentration in the fermentation broth of greater than or equal to 8 mM; and maintaining the alcohol concentration in the fermentation broth at less than 100 mM throughout the fermentation.

In order to provide a free acid concentration of greater than or equal to 8 mM, the control method according to the embodiment comprises steps S100 – S500 in Figure 1. At step S100, the total acid concentration of said one or more organic acids in the fermentation broth is determined. This can be conducted, for example, by obtaining a sample of the fermentation broth and measuring the total acid concentration of said one or more organic acids in the sample, e.g. using HPLC. At step S200, the pH of the fermentation broth is determined (e.g. with inline measurement of the fermentation, or with measurement of the sample). It can be appreciated that steps S100 and S200 may be conducted concurrently or sequentially in either order. Subsequently, at step S300, the free acid concentration in the fermentation broth is determined based on the values from steps S100 and S200 (e.g. in accordance with Equation 2 above). Alternative methods of determining the free acid concentration in the fermentation broth may be used in place of steps S100 – S300.

Subsequently, at step S400, a target pH of the fermentation broth is determined, for example based on the total acid concentration of the one or more organic acids, the pK_a value of the acids (which is weakly temperature dependent), and the set point free acid concentration (i.e. a value greater than 8 mM, e.g. 10 mM). The set point free acid concentration is selected such that the organic acid-producing microorganism is not inhibited, whilst still inhibiting contaminant microorganisms. It can be understood that the target pH will change during the course of the fermentation (e.g. where the set point free acid concentration remains constant and the total acid concentration of the organic acids in the fermentation broth changes).

Subsequently, at step S500, the pH of the fermentation is varied to reach the target pH determined at step S400. The control of the pH may be conducted by one or more of: addition of exogenous base to the fermentation broth to increase the pH, allowing the fermentation to continue producing the one or more organic acids to reduce the pH, and addition of exogenous acid to the fermentation broth to reduce the pH.

Steps S100 – S500 are repeated during the fermentation process in order to maintain a free acid concentration of greater than or equal to 8 mM in the fermentation broth.

It can be appreciated that during the lag-phase and possibly at least part of the exponential growth phase of the fermentation process, the total acid concentration of the one or more organic acids in the

fermentation broth may be insufficient to provide a free acid concentration of greater than or equal to 8 mM, for example, the total acid concentration may be less than 8 mM, or the pH required to provide an free acid concentration greater than or equal to 8 mM may be lower than the organic acid-producing microorganism can withstand without becoming inhibited (e.g. below pH 3). Accordingly, the control method may not be implemented on starting the fermentation, for example, the control method may be implemented once the total acid concentration of the fermentation broth exceeds 8 mM.

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The features disclosed in the foregoing description, or in the following claims, or in the accompanying drawings, expressed in their specific forms or in terms of a means for performing the disclosed function, or a method or process for obtaining the disclosed results, as appropriate, may, separately, or in any combination of such features, be utilised for realising the invention in diverse forms thereof.

While the invention has been described in conjunction with the exemplary embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the invention set forth above are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of the invention.

For the avoidance of any doubt, any theoretical explanations provided herein are provided for the purposes of improving the understanding of a reader. The inventors do not wish to be bound by any of these theoretical explanations.

Any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word “comprise” and “include”, and variations such as “comprises”, “comprising”, and “including” will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent “about,” it will be understood that the particular value forms another embodiment. The term “about” in relation to a numerical value is optional and means for example +/- 10%.

Examples

Figures 2 to 4 provide the results of an experiment conducted in controlling a thermophilic fermentation of a *Moorella thermoacetica* microorganism producing acetic acid on a substrate of H_2 and CO_2. The data presented in Figures 2 to 4 is provided below in Tables 1 and 2.

Experimental Method

The reactor used in this experiment was a demonstration-scale gas fermentation reactor, with a nominal volume of approximately 5000 L. The reactor was operated by introducing a technical grade gas mixture (80% H_2 and 20% CO_2). The reactor was operated in batch mode, repressurizing twice per day to 2.5 bar overpressure after consumption of gas. The temperature of the fermentation broth was maintained at the thermophilic setpoint through an internal jacket. Mixing was achieved with an external recirculation loop pumping liquid volume at approximately 40 L/minute.

The initial pH of the fermentation broth was below 6. Readings of pH were taken through an inline pH probe, and they were confirmed with offline pH measurements. Measurements of the total acetic acid concentration extracellularly in the fermentation broth (i.e. including free acetic acid concentration, $[CH_3COOH]$ and acetate concentration, $[CH_3COO^-]$) were made from a sample of the fermentation broth using a Shimadzu high performance liquid chromatography instrument (Bio-Rad HPX-7H column). By using the total acetic acid concentration and the sample pH, the concentration of free acetic acid, $[CH_3COOH]$, and the concentration of acetate, $[CH_3COO^-]$, in the fermentation broth could be determined. Inline measurements of the gas composition were made with a Prima BT mass spectrometer (Thermo Scientific, Carlsbad, CA).

The reactor contained semi-defined fermentation medium. After removing residual oxygen by sparging with technical-grade CO_2 to a pressure of 2.5 bar, the initial volume was inoculated with preculture. That preculture had been grown anaerobically, in a semi-defined medium under controlled lab conditions. Samples were taken from the reactor every few hours from a hand valve in the recirculation line.

The composition of the gas in the fermenter was adjusted to 80 : 20 H_2 : CO_2 to provide the fermentation substrate. Consumption of gas sparged into the reactor was indicated by a decrease in pressure of the reactor and a change in partial pressure of H_2 and CO_2 measured by an inline gas analyser. The presence of autotrophic metabolism was indicated by the increase of organic acid concentration in the fermentation broth (quantified by HPLC), as well as by the decrease in H_2 and CO_2 quantified by offline mass spectrometer (MS) measurements. Likewise, the presence of methanogens was suggested by the detection of CH_4 in the headspace of the reactor (quantified by offline MS). Changes in the relative abundance of *Moorella thermoacetica* and other, contaminant, organisms were quantified by Sanger and Illumina sequencing of DNA extracted from biomass recovered from the fermentation broth sampled at various timepoints throughout the fermentation.

Over the course of the fermentation, the concentration of free acetic acid in the fermentation broth was calculated at each sample point, using the measured concentration of acetic acid and the measured pH value at that point. This relationship is a well-established correlation based on the pK_a of the acetic acid/acetate pair (see Equation 2 above).

Figures 2 and 3 illustrate the impact of controlling the free acetic acid concentration in the fermentation on the relative abundance of contaminants in the fermentation and the resulting impact on CH_4 production by the fermentation.

At a fermentation time of 80 hours, free acetic acid concentration was 0.7 mM from a total acetic acid concentration of 10.6 mM, low relative to the concentration of around 8 mM at which inhibition of contaminant microorganisms occurs. This was due to the low relative abundance (9.3%) of the *Moorella thermoacetica* strain producing the acetic acid compared to other thermophilic strains in the fermentation broth, including *Methanothermobacter*. As the fermentation progressed, the *Moorella thermoacetica* began to grow at a greater rate than the contaminant microorganisms, reaching a relative abundance of 13.9% after 141 hours of fermentation and with a resulting gradual increase in free acetic acid concentration to 10 mM from a total acetic acid concentration of 41 mM. However, at this stage, CH_4 concentration in the off-gas remained high at 21%, and the relative abundance of *Methanothermobacter* had also increased to 16.2%. Over the next 120 hours of the fermentation, the free acetic acid concentration was kept roughly in the range of 8-10 mM; an increase in the relative abundance of *Moorella thermoacetica*, (to 27%) and a large decrease in the concentration of CH_4 in the off-gas (to 2.5%) was observed. At this point, the relative abundance of *Methanothermobacter* remained high (21.9% after 236 hours), but the relative abundance of other strains had fallen away (down from 69.6% after 141 hours to 51.1% after 236 hours). Subsequently, with increasing relative abundance of *Moorella thermoacetica*, the free acetic acid concentration and total acetic acid concentration rose further, reaching 23 mM and 82 mM, respectively, after 292 hours, and 31 mM and 104 mM, respectively, after 310 hours. The further increases in free acetic acid concentration were accompanied by a further decrease in CH_4 concentration in the off-gas (down to 1.1% after 310 hours) and moreover a decrease in the relative abundance of *Methanothermobacter* (down to 10.4% after 292 hours, and 9.7 % after 357 hours) as well as a decrease in the relative abundance of other contaminant strains (down to 32.2 % after 292 hours, and 24.4 % after 357 hours).

Throughout the fermentation, ethanol was not detected.

Table 1

Time / h	Methane / %	Total acetic acid / mM	*Moorella thermoacetica* / %	*Methano-thermo-bacter* / %	Other strains / %	Free acetic acid / mM
71	-	10.6	-	-	-	0.6
80	-	14.6	9.3	9.2	81.5	0.7
95	-	17.8	-	-	-	3.3
101	-	17.8	-	-	-	2.5
101	-	24.8	-	-	-	3.6
117	-	24.7	-	-	-	4.9
123	-	41.3	-	-	-	9.5
141	21	41.2	13.9	16.2	69.9	10.6
149	-	34.0	-	-	-	9.1

149	-	42.0	-	-	-	11.2
166	21.8	42.1	-	-	-	8.6
190	-	39.6	4.5	31.9	63.6	8.5
236	-	46.0	27	21.9	51.1	12.3
261	2.5	60.2	-	-	-	16.6
292	2.8	81.9	57.4	10.4	32.2	23.6
310	1.1	104.3	-	-	-	31.0
357	-	-	65.9	9.7	24.4	-

Figure 4 illustrates the trend between the concentration of free acetic acid in the fermentation broth and the percentage of methane in the off-gas from the fermentation. The data in Figure 4 and Table 2 was obtained from the same fermentation as for the data in Figures 2 and 3 and Table 1. The fermentation was run beyond the first 357 hours covered by Table 1, up to 1965 hours, and off gas CH_4 concentration and free acetic acid concentration measurement continued.

Figure 4 illustrates that a transition in methanogen activity within the fermentation occurs at a free acetic acid concentration of around 8 mM: below this threshold, methanogen growth and activity is not (substantially) inhibited, but above 8 mM there is a sharp drop in their growth and activity, which is reflected both by the decreased CH_4 concentration in the off-gas, as shown in Figure 4, and also the decrease in their relative abundance, as shown in Figure 3 and Table 1.

Once a free acetic acid concentration of above 15 mM is reached, there is little-to-no correlation between the free acetic acid concentration and the concentration of CH_4 in the off-gas from the fermentation. However, the free acetic acid concentration may have an influence on the growth of the organic acid-producing microorganism (i.e. *Moorella thermoacetica*) and facilitates operation of the fermentation process at the desired pH even as total acid concentration of said one or more organic acids in the fermentation broth continues to increase due to growth and activity of the organic acid-producing microorganism.

Table 2

Free acetic acid / mM	CH4 / %
8.6	21.8
10.6	21
10.6	3.7
12.6	2.5
15.4	0.0053

16.0	0.8674
16.1	0.94
16.7	1.195
17.7	0.72
18.3	0.84
18.3	1.069
18.9	0.719
20.0	0.6151
20.3	1.11
20.6	0.9138
21.5	0.8
22.6	0.9018
22.7	0.959
23.5	2.8
24.9	1.158
24.9	1.0076
27.6	0.222
31.0	1.1
37.7	1.2416

References

A number of publications are cited above in order to more fully describe and disclose the invention and the state of the art to which the invention pertains. Full citations for these references are provided below. The entirety of each of these references is incorporated herein.

Zhang et al., 2018, Free acetic acid as the key factor for the inhibition of hydrogenotrophic methanogenesis in mesophilic mixed culture fermentation, Bioresource Technology 264 (2018) 17-13

Diender et al., 2023, The role of ethanol oxidation during carboxydotrophic growth of Clostridium autoethanogenum, Microbial Biotechnology, 2023; 16:2082-2093.

For standard molecular biology techniques, see Sambrook, J., Russel, D.W. *Molecular Cloning, A Laboratory Manual.* 3 ed. 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Claims:

1. A method of controlling a microbial fermentation of a microorganism in a fermentation broth, said microorganism being capable of converting one or more gases into one or more organic acids, wherein:

said microorganism is thermophilic; and

the method comprises:

varying the pH of the fermentation broth such as to provide a free acid concentration in the fermentation broth of greater than 8 mM; and

maintaining the alcohol concentration in the fermentation broth at less than 100 mM throughout the fermentation.

2. The method according to claim 1, wherein the method further comprises maintaining the temperature of the fermentation broth above 45°C.

3. The method according to claim 1 or 2, wherein said microorganism is selected from the group consisting of:

thermophilic acetogens;

thermophilic propionibacteria;

thermophilic butyric acid bacteria;

thermophilic lactic acid bacteria;

thermophilic succinic acid bacteria; and

thermophilic hydrogen oxidising bacteria.

4. The method according to claim 3, wherein said microorganism is selected from the group consisting of: *Moorella, such as Moorella thermoaceticum, Thermoanaerobacter, Thermoanaerobacterium, Acetogenum, Carboxydothermus*, or *Acetobacterium*.

5. The method according to claim 4, wherein said microorganism comprises genes encoding an alcohol dehydrogenase (AdhE) or acetaldehyde oxidoreductase (AOR).

6. The method according to any one of the preceding claims, wherein the method comprises maintaining the concentration of said one or more organic acids in the fermentation broth of greater than 80 mM.

7. The method according to any one of the preceding claims, wherein the total organic acid productivity of the microbial fermentation is greater than or equal to 8 mM/hour

8. The method according to any one of the preceding claims, wherein said one or more organic acids are selected from the group consisting of:

acetic acid;

butyric acid;

propionic acid;

lactic acid; and
succinic acid.

9. The method according to claim 8, wherein said one or more organic acids consists of acetic acid and the method comprises maintaining the ethanol concentration in the fermentation broth at less than 100 mM throughout the fermentation .

10. The method according to any one of the preceding claims, wherein the total alcohol productivity of the microbial fermentation is less than or equal to 1 mM/hour).

11. The method according to any one of the preceding claims, wherein said microbial fermentation is an industrial scale process.

12. The method according to claim 11, wherein said industrial scale process is a fermentation in at least 1 m^3 scale, at least 5 m^3 scale, at least 50 m^3 scale, at least 150 m^3 scale, or at least 250 m^3 scale.

13. The method according to any preceding claim, wherein the method comprises maintaining the fermentation broth at a pH less than 7.

14. The method according to any one of the preceding claims, wherein the method comprises:
determining the free acid concentration or total acid concentration of the one or more organic acids;
determining a target pH of the fermentation broth; and
varying the pH of the fermentation broth to reach the target pH.

15. A microbial fermentation of a microorganism in a fermentation broth, said microorganism being capable of converting one or more gases into one or more organic acids, wherein:
said microorganism is thermophilic;
the alcohol concentration in the fermentation broth is less than 100 mM; and
the free acid concentration in the fermentation broth is greater than or equal to 8 mM.