

CONTROL OF CARBON DIOXIDE LEVELS AND pH IN SMALL VOLUME REACTORS

RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Patent Application Serial No. 61/719,027, filed October 26, 2012, and entitled "Control of Carbon Dioxide Levels and pH in Small Volume Reactors"; U.S. Provisional Patent Application Serial No. 61/869,111 filed August 23, 2013, and entitled "Control of Carbon Dioxide Levels and pH in Small Volume Reactors"; and European Application No. 13306462.6, filed
10 October 23, 2013, and entitled "Control of Carbon Dioxide Levels and pH in Small Volume Reactors," each of which is incorporated herein by reference in its entirety for all purposes.

TECHNICAL FIELD

15 Systems and methods for the control of carbon dioxide levels and pH within small volume reactors are generally described.

BACKGROUND

 There is currently a great deal of interest in developing small volume bioreactors
20 for growing cells, for example, for biopharmaceutical production. Controlling carbon dioxide levels and pH in such reactors can be challenging. Even small amounts of acid, base, and/or carbon dioxide to a small-scale bioreactor can lead to large relative shifts in carbon dioxide levels and/or pH, which can adversely impact bioreactor operation. Improved systems and methods for controlling carbon dioxide levels and pH in such
25 reactors are therefore desirable.

SUMMARY

 Control of carbon dioxide levels and pH within small volume reactors, as well as related
30 systems and methods, are generally described. In certain ~~embodiments~~examples, control of carbon dioxide levels and pH within liquid growth medium within a bioreactor, such as a reactor configured to grow one or more types of biological cells, is described. The subject matter of the present ~~invention~~disclosure involves, in some cases, interrelated products,

alternative solutions to a particular problem, and/or a plurality of different uses of one or more systems and/or articles. In one aspect, the present invention refers to a bioreactor system, comprising a reactor chamber having a volume of equal to or less than about 50 milliliters and containing a liquid growth medium including at least one biological cell and a buffer and a gaseous headspace containing carbon dioxide above the liquid growth medium; a first inlet connecting a source of carbon dioxide gas to the gaseous headspace; and a second inlet connecting a source of an alkaline liquid to the liquid growth medium, wherein use of the bioreactor system can be used to achieve a $K_L a$ of carbon dioxide between the gaseous headspace and the liquid growth medium of at least about 0.1 hour^{-1} to less than 15 hours^{-1} . In another aspect, the present invention refers to a bioreactor system, comprising a reactor chamber having a volume of equal to or less than about 50 milliliters and containing a liquid growth medium including at least one biological cell and a buffer and a gaseous headspace containing carbon dioxide above the liquid growth medium; a first inlet connecting a source of carbon dioxide gas to the gaseous headspace; and a sensor within the reactor chamber configured to determine the concentration of carbon dioxide and/or pH within the liquid growth medium, wherein use of the bioreactor system can be used to achieve a $K_L a$ of carbon dioxide between the gaseous headspace and the liquid growth medium of at least about 0.1 hour^{-1} to less than 15 hours^{-1} .

In one aspectexample, a bioreactor system is provided. In certain embodimentsexamples, the bioreactor system comprises a reactor chamber having a volume of equal to or less than about 50 milliliters and containing a liquid growth medium including at least one biological cell and a buffer and a gaseous headspace containing carbon dioxide above the liquid growth medium, a first inlet connecting a source of carbon dioxide gas to the gaseous headspace, and a second inlet connecting a source of an alkaline liquid to the liquid growth medium.

In some embodimentsexamples, the bioreactor system comprises a reactor chamber having a volume of equal to or less than about 50 milliliters and containing a liquid growth medium including at least one biological cell and a buffer and a gaseous headspace containing carbon dioxide above the liquid growth medium, a first inlet connecting a source of carbon dioxide gas to the gaseous headspace, and a sensor within the reactor chamber configured to determine the concentration of carbon dioxide and/or pH within the liquid growth medium.

According to certain embodimentsexamples, a method of operating a bioreactor is described. In some embodimentsexamples, the method comprises providing a reactor chamber having a volume of equal to or less than about 50 milliliters and containing a liquid growth medium including at least one biological cell and a gaseous headspace containing carbon dioxide above the liquid growth medium, and operating the reactor such that the $k_L a$ of carbon dioxide between the headspace and the bulk of the liquid medium is at least about 0.1 hours^{-1} and less than about

15 hours⁻¹. In one aspect, the present invention refers to a method of operating a bioreactor, comprising providing a reactor chamber having a volume of equal to or less than about 50 milliliters and containing a liquid growth medium including at least one biological cell and a gaseous headspace containing carbon dioxide above the liquid growth medium; and operating the reactor such that the $k_L a$ of carbon dioxide between the headspace and the bulk of the liquid medium is at least about 0.1 hours⁻¹ and less than about 15 hours⁻¹. In another aspect, the present invention refers to a method of operating a bioreactor, comprising providing a reactor chamber having a volume of equal to or less than about 50 milliliters, the reactor chamber containing a liquid growth medium including at least one biological cell, and a gaseous headspace containing carbon dioxide above the liquid growth medium; transporting a gas containing carbon dioxide to the gaseous headspace; and transporting an alkaline liquid to the liquid growth medium, wherein the method results in a $k_L a$ of carbon dioxide between the gaseous headspace and the liquid growth medium is at least about 0.1 hours⁻¹ to less than about 15 hours⁻¹.

In certain ~~embodiment~~examples, the method comprises providing a reactor chamber having a volume of equal to or less than about 50 milliliters. The reactor chamber contains, in some ~~embodiment~~examples, a liquid growth medium including at least one biological cell, and a gaseous headspace containing carbon dioxide above the liquid growth medium. In some ~~embodiment~~examples, the method comprises transporting a gas containing carbon dioxide to the gaseous headspace, and transporting an alkaline liquid to the liquid growth medium.

Other advantages and novel features of the present invention will become apparent from the following detailed description of various non-limiting embodiments of the invention when considered in conjunction with the accompanying figures. In cases

CLAIMS

1. A bioreactor system, comprising:

5 a reactor chamber having a volume of equal to or less than about 50 milliliters and containing a liquid growth medium including at least one biological cell and a buffer and a gaseous headspace containing carbon dioxide above the liquid growth medium;

a first inlet connecting a source of carbon dioxide gas to the gaseous headspace; and

a second inlet connecting a source of an alkaline liquid to the liquid growth medium,

10 wherein use of the bioreactor system can be used to achieve a $K_L a$ of carbon dioxide between the gaseous headspace and the liquid growth medium of at least about 0.1 hour^{-1} to less than 15 hours^{-1} .

2. A bioreactor system, comprising:

15 a reactor chamber having a volume of equal to or less than about 50 milliliters and containing a liquid growth medium including at least one biological cell and a buffer and a gaseous headspace containing carbon dioxide above the liquid growth medium;

a first inlet connecting a source of carbon dioxide gas to the gaseous headspace; and

a sensor within the reactor chamber configured to determine the concentration of carbon

20 dioxide and/or pH within the liquid growth medium, wherein use of the bioreactor system can be used to achieve a $K_L a$ of carbon dioxide between the gaseous headspace and the liquid growth medium of at least about 0.1 hour^{-1} to less than 15 hours^{-1} .

3. The bioreactor system of claim 1, comprising a sensor within the reactor chamber

25 configured to determine the concentration of carbon dioxide within the liquid growth medium.

4. The bioreactor system of any one of claims 1-3, wherein an aspect ratio of the reactor chamber is less than about 10.

30 5. The bioreactor system of any one of claims 2-4, wherein the sensor is in direct contact with the liquid growth medium.

6. The bioreactor system of any one of claims 1-5, wherein the reactor chamber comprises a moveable wall.

providing a reactor chamber having a volume of equal to or less than about 50 milliliters and containing a liquid growth medium including at least one biological cell and a gaseous headspace containing carbon dioxide above the liquid growth medium; and

operating the reactor such that the $k_L a$ of carbon dioxide between the gaseous headspace and the bulk of the liquid growth medium is at least about 0.1 hours⁻¹ and to less than about 15 hours⁻¹.

28. A method of operating a bioreactor, comprising:

providing a reactor chamber having a volume of equal to or less than about 50 milliliters, the reactor chamber containing:

a liquid growth medium including at least one biological cell, and

a gaseous headspace containing carbon dioxide above the liquid growth medium;

transporting a gas containing carbon dioxide to the gaseous headspace; and

transporting an alkaline liquid to the liquid growth medium, wherein the method results in a $k_L a$ of carbon dioxide between the gaseous headspace and the liquid growth medium is at least about 0.1 hours⁻¹ to less than about 15 hours⁻¹.

~~29. The method of claim 28, comprising operating the reactor such that the $k_L a$ of carbon dioxide between the headspace and the bulk of the liquid medium is at least about 0.1 hours⁻¹ and less than about 15 hours⁻¹.~~

~~30. 29.~~ The method of ~~any one of claims 27-29~~ claim 27 or 28, comprising transporting an acidic material to the liquid growth medium.

~~31. 30.~~ The method of ~~any one of claims 28-29~~ claim 28, wherein the osmolarity of the liquid growth medium is substantially constant during the step of transporting the gas.

~~32. 31.~~ The method of any one of claims 28-~~31~~ 28-31, wherein the alkaline liquid contains bicarbonate ions.

~~33. 32.~~ The method of any one of claims 27 and ~~29-32~~ 29-31, wherein the $k_L a$ of carbon dioxide between the headspace and the bulk of the liquid medium is less than or equal to about 15 hour⁻¹.

34.33. The method of any one of claims ~~27-33~~27-32, wherein the reactor chamber contains a volume of the liquid medium of equal to or greater than 10 microliters and less than about 50 milliliters.