

**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 systems and methods, are generally described. In one aspect, the present disclosure  
30   refers to a method of producing a recombinant protein, the method comprising: (a) providing a bioreactor system, comprising: a reactor chamber having a volume of equal to or less than about 50 milliliters containing a liquid growth medium including at least one mammalian cell that produces a recombinant protein and a liquid growth medium and a

gaseous head space containing oxygen and carbon dioxide above the liquid growth medium; a first inlet connecting (i) a gas manifold connected to a source(s) of carbon dioxide gas and oxygen to (ii) the gaseous headspace; a second inlet connecting a first reservoir comprising sterile water to the liquid growth medium; a third inlet connecting a second reservoir comprising an alkaline liquid to the liquid growth medium; a dissolved carbon dioxide sensor disposed in the liquid growth medium; an oxygen sensor disposed in the liquid growth medium; a pH sensor disposed in the liquid growth medium; and optionally, a gas-permeable membrane that separates the gaseous headspace and the liquid growth medium, and/or a fourth inlet connecting a third reservoir comprising a feed culture medium to the liquid growth medium, and (b) operating the reactor under conditions that allow for the production of the recombinant protein by the at least one biological cell, and the achievement of a  $K_La$  of carbon dioxide between the headspace and the bulk of the liquid growth medium of at least about 0.1 hours<sup>-1</sup> and less than about 15 hours<sup>-1</sup>.

In certain 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 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 example, a bioreactor system is provided. In certain examples, 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 examples, 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 examples, a method of operating a bioreactor is described. In some examples, 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  
5 above the liquid growth medium, and operating the reactor such that the  $k_{La}$  of carbon dioxide between the headspace and the bulk of the liquid medium is at least about 0.1 hours<sup>-1</sup> and less than about 15 hours<sup>-1</sup>.

In certain 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  
10 some 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 examples, the method comprises transporting a gas containing carbon dioxide to the gaseous head space, and transporting an alkaline liquid to the liquid growth medium.

Other advantages and novel features of the present disclosure will become  
15 apparent from the following detailed description of various non-limiting examples of the disclosure when considered in conjunction with the accompanying figures. In cases where the present specification and a document incorporated by reference include conflicting and/or inconsistent disclosure, the present specification shall control.

## 20                    **BRIEF DESCRIPTION OF THE DRAWINGS**

Non-limiting embodiments of the present invention will be described by way of example with reference to the accompanying figures, which are schematic and are not intended to be drawn to scale. In the figures, each identical or nearly identical component illustrated is typically represented by a single numeral. For purposes of  
25 clarity, not every component is labeled in every figure, nor is every component of each embodiment of the invention shown where illustration is not necessary to allow those of ordinary skill in the art to understand the invention. In the figures:

FIG. 1 is a cross-sectional schematic illustration of a reactor system, according to one set of embodiments;

30                    FIGS. 2A-2C are, according to certain embodiments, cross-sectional schematic illustrations of a reactor chamber and a mode of operating the same;

FIG. 3 is a bottom-view cross sectional schematic illustration of a reactor system including a plurality of reactor chambers arranged in series, according to some embodiments;

5        FIG. 4 is a cross-sectional schematic illustration of a reactor system, according to certain embodiments;

FIG. 5 is a cross-sectional schematic illustration of a gas manifold for a reactor system, according to one set of embodiments;

FIG. 6 is a cross-sectional schematic illustration of a gas manifold for a reactor system, according to some embodiments;

10        FIG. 7 is a photograph of a reactor system, according to certain embodiments;

FIG. 8 is a plot of phase difference versus frequency, according to one set of embodiments;

FIG. 9 is a plot of phase difference versus modulation frequency, according to some embodiments;

15        FIG.10 is a calibration plot for carbon dioxide, according to certain embodiments;

FIG. 11 is a gas transfer plot obtained using an oxygen sensor, according to one set of embodiments;

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**CLAIMS**

1. A method of producing a recombinant protein, the method comprising:
  - (a) providing a bioreactor system, comprising:
    - 5 a reactor chamber having a volume of equal to or less than about 50 milliliters containing a liquid growth medium including at least one mammalian cell that produces a recombinant protein and a liquid growth medium and a gaseous head space containing oxygen and carbon dioxide above the liquid growth medium;
    - a first inlet connecting (i) a gas manifold connected to a source(s) of carbon dioxide gas and oxygen to (ii) the gaseous headspace;
    - 10 a second inlet connecting a first reservoir comprising sterile water to the liquid growth medium;
    - a third inlet connecting a second reservoir comprising an alkaline liquid to the liquid growth medium;
    - 15 a dissolved carbon dioxide sensor disposed in the liquid growth medium;
    - an oxygen sensor disposed in the liquid growth medium;
    - a pH sensor disposed in the liquid growth medium; and
    - optionally, a gas-permeable membrane that separates the gaseous headspace and the liquid growth medium, and/or a fourth inlet connecting a third reservoir comprising a feed culture medium to the liquid growth medium, and
    - 20 (b) operating the reactor under conditions that allow for the production of the recombinant protein by the at least one biological cell, and the achievement of a  $K_{La}$  of carbon dioxide between the headspace and the bulk of the liquid growth medium of at least about  $0.1 \text{ hours}^{-1}$  and less than about  $15 \text{ hours}^{-1}$ .
- 25 2. The method of claim 1, wherein the bioreactor system comprises a gas permeable membrane that separates the gaseous headspace and the liquid growth medium.
3. The method of claim 1, wherein the bioreactor system comprises a fourth inlet  
30 connecting a third reservoir comprising a feed culture medium to the liquid growth medium.
4. The method of claim 1, wherein the bioreactor system comprises a gas-permeable membrane that separates the gaseous headspace and the liquid growth medium, and a fourth

inlet connecting a third reservoir comprising a feed culture medium to the liquid growth medium.

5        5.        The method of any one of claims 1-4, wherein the reactor chamber has an aspect ratio of less than about 10.

6.        The method of any one of claims 1-5, wherein the mammalian cell is selected from the group consisting of: a primate cell, a bovine cell, a horse cell, a porcine cell, a goat cell, dog cells, a cat cell, a human cell, and a hamster cell.

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7.        The method of any one of claims 1-6, wherein the mammalian cell is a cardiac cell, a fibroblast, a keratinocyte, a hepatocyte, a chondrocyte, a neural cell, an osteocyte, a muscle cell, a blood cell, an endothelial cell, an immune cell, or a stem cell.

15        8.        The method of any one of claims 1-7, wherein the mammalian cell is a genetically engineered cell.

9.        The method of any one of claims 1-8, wherein the gas manifold is connected to a source of carbon dioxide gas, and the source of carbon dioxide gas comprises substantially  
20        pure carbon dioxide.

10.       The method of any one of claims 1-9, wherein the alkaline liquid contains bicarbonate ions.

25        11.       The method of any one of claims 1-10, wherein the alkaline liquid has a pH of greater than or equal to 7.5.

12.       The method of any one of claims 1-11, wherein the bioreactor system further comprises a fifth inlet connecting a fourth reservoir comprising an acidic material to the  
30        liquid growth medium.

13.       The method of claim 12, wherein the acidic material has a pH of less than or equal to 6.5.

14. The method of any one of claims 1-13, wherein operation of the bioreactor system in step (b) results in a partial pressure of carbon dioxide above the liquid growth medium of between about 0% and about 20%.

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15. The method of any one of claims 1-14, wherein the operation of the bioreactor system in step (b) results in a total pressure in the gaseous headspace of about 0 psi to about 15 psi.

10 16. The method of claim 1, wherein the gaseous headspace and the liquid growth medium are in direct contact.

17. The method of any one of claims 1-16, wherein the recombinant protein is an antibody.

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18. The method of any one of claims 1-17, wherein the at least one mammalian cell is a Chinese Hamster Ovary (CHO cell).

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**CONTROL OF CARBON DIOXIDE LEVELS AND PH IN SMALL VOLUME REACTORS**

**Abstract**

Strategies to control the level of dissolved carbon dioxide (CO<sub>2</sub>) concentrations  
5 and/or pH in small volume reactor chambers, and associated articles, systems, and  
methods, are generally provided. In certain embodiments, the reactor chambers can be  
configured to contain at least one biological cell.

Figure 1