need for specially-designed bags to accommodate the pH and DO probes, increase the operational cost and complexity of this system. In addition, the base addition required to raise culture pH to the defined setpoint in pH-controlled bioreactors increases the culture osmolality. Depending on the extent of the osmolality increase in the bioreactor, the associated decrease in cell growth and viability (deZengotita et al., 2002; Zhu et al., 2005) may offset the benefits of pH control. In addition, if the pH probe malfunctions, the resulting pH perturbations may alter cell metabolism and promote cell death (Miller et al., 1988; Osman et al., 2002).

Tight pH and DO controls may not be necessary for certain cell culture applications, such as, for example, the routine passage of cells in small-scale culture systems, such as shake flasks and spinners, for cell maintenance and expansion. However, pH and DO extremes are detrimental to cell growth and viability (Lin et al., 1993; Link et al., 2004; Miller et al., 1988; Osman et al., 2001), and may affect product quality (Restelli et al., 2006; Yoon et al., 2005). Therefore, it is critically important to maintain some control over such growth conditions of cells for all stages of biologics manufacturing. Researchers previously demonstrated success in culturing CHO cells in a pH range of 6.8-7.3 and in the DO range of 10-100% of air saturation (Link *et al.* 2004; Restelli *et al.* 2006; Trummer *et al.* 2006; Yoon *et al.* 2005).

The added features of conventional bioreactors such as real-time pH monitors and DO monitoring control add significantly to the cost and labor-intensity of cell culture in biological manufacturing. Further, the failure or malfunction of these features can cause unacceptable variations and potential loss of the cell culture which is very costly in time and resources.

Thus, there is a need for improved methods for culturing eukaryotic cells without the need for introduction of strong bases, and without additional monitoring and real-time control of pH and DO.

SUMMARY ~~OF THE INVENTION~~

The ~~invention~~disclosure provides an apparatus and method to maintain pH in a cell culture system without the addition of base. In a bicarbonate-containing cell culture medium, the amount of CO_2 in the medium affects the pH of the medium, based on the carbonic acid-bicarbonate buffer equilibrium (Equation 1):

$$CO_2 + HOH <====> H_2CO_3 <====> H^+ + HCO_3^-$$

$$pH = pK - \log([CO_2]/[HCO_3^-])$$

Thus, the ~~invention~~disclosure exploits this relationship to adjust the pH of cell culture medium without the need for addition of strong acids or bases by increasing or decreasing the dissolved CO_2 concentration using the dynamic interface of a liquid phase and gas phase of a cell culture system. The ~~invention~~disclosure provides a method for achieving this modulation and an apparatus for practicing the method.

In general, the apparatus of the ~~invention~~disclosure is supplied with air, oxygen or a combination of these gases to maintain the dissolved oxygen of the cell culture. By providing a gas mixture (which may be manipulated in terms of its composition and the rate of introduction) to the head space of the apparatus, CO_2 can either be added to or removed from the cell culture medium depending on the differential concentration of CO_2 between the liquid and gas phase. The removal of CO_2 from the head space will increase the culture pH as dissolved CO_2 in the medium will diffuse out into the head space. Conversely, when CO_2 is added to the apparatus at a concentration that is higher than that of the medium, the CO_2 will dissolve into the medium and the culture pH will decrease. This ~~invention~~disclosure provides a method that allows CO_2 transfer into and out of the cell culture to maintain culture pH without addition of base.

In one aspect, there is provided a method for batch culturing eukaryote cells comprising:

providing cell culture inoculant comprising eukaryotic cells in a bicarbonate-containing culture liquid to a vessel,

said vessel having walls that encapsulate said cell culture and a gas phase head space above said cell culture, and wherein said vessel comprises at least one port that provides an entrance and an egress of gas to and from said head space;

agitating said vessel; and

providing gas to said head space through said port wherein said gas contains an amount of CO_2, and wherein said amount of CO_2 in said gas is modulated over time to adjust the pH of said cell culture in order to maintain a predetermined pH of said cell culture.

wherein said CO_2 is provided in an amount of 8% (v/v) of said gas on day 1, in an amount of 5% (v/v) of said gas on day 2, and in an amount of 2% (v/v) of said gas thereafter.

In one aspect, there is provided a method for batch culturing eukaryote cells comprising:

providing cell culture comprising eukaryotic cells in a bicarbonate-containing culture liquid to a vessel,

said vessel having walls that encapsulate said cell culture and a gas phase head space above said cell culture, and wherein said vessel comprises at least one port that provides an entrance and an egress of gas to and from said head space;

agitating said vessel; and

providing gas to said head space through said port wherein said gas contains 8% CO_2 (v/v) of gas during the first day, 5% CO_2 (v/v) of gas the second day and 2% CO_2 (v/v) of gas thereafter.

Thus, the ~~invention~~disclosure provides a method for culturing eukaryotic cells comprising eukaryotic cells in a bicarbonate-containing culture liquid in a vessel, wherein the vessel has walls that encapsulate the cell culture and a gas phase head space above said cell culture. The vessel also comprises at least one port that provides an entrance and an egress of gas from said head space. The vessel is agitated to provide a dynamic interface between the liquid phase and the gas phase. The pH of the culture may be monitored and a gas is provided to the head space through said port wherein the gas contains an amount of CO_2 to effect a decrease in the pH as more CO_2 dissolves into the cell culture, or accumulated CO_2 is removed from the head space through the port to effect an increase in the pH of the cell culture. The pH is thus maintained at a predetermined range.

Generally, the partial pressure of dissolved CO_2 in the cell culture medium is maintained at an amount of 1 to 200 mmHg. In some embodiments the partial pressure of dissolved CO_2 is 10 to 180 mmHg. In some embodiments the partial

16°. In other embodiments, the rock angle is between 7-16°. In other embodiments, the rock angle is between 8-12°.

In some embodiments, the rock rate is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,1 12, 13, 14 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, ,34, 35, 36, 37, 38, 39, 40 rpm. In some embodiments, the rock rate is between 19-25 rpm. In some embodiments, the rock rate is between 20-24 rpm. In some embodiments, the rock rate is between 21-23 rpm.

The method may be performed in which the vessel contains a single port that allows ingress and egress of gas from the head space of the culture. Alternatively, the vessel may contain a plurality of ports.

The pH of the culture is monitored either continuously or intermittently and gas is infused into the head space such that the CO_2 level of the gas in the head space is provided either to increase or decrease the concentration of dissolved CO_2 in the liquid phase of the culture such that the pH of the liquid phase is adjusted to a predetermined value.

In an alternative embodiment, the method may include a step of perfusing fresh culture medium into the cell culture through a medium port. The fresh medium has a pH that provides adjustment of the overall pH of the cell culture upon addition such that the pH of the fresh medium is partially maintaining the cell culture at a predetermined pH range. The modulation of pH using fresh medium having a predetermined pH is helpful in the culture method, but is not sufficient to completely control the pH.

The method is adaptable for any size culture. In some embodiments, the method is performed in disposable bioreactor bags which are available commercially. Such bioreactor bags are available in such volumes as 500 mL, 1 L, 2 L, 10 L, 20 L, 50 L, 100 L, 200 L, 500 L, and 1000 L.

Various parameters of the culture may be monitored and controlled. Such parameters may be controlled in an automated process as calculations are performed by a computer. Some parameters that may be controlled, alone or in combination, include, but are not limited to, gas flow, pH, dissolved CO_2 concentration, temperature, and agitation.

The ~~invention~~disclosure also provides an apparatus for performing the method of the ~~invention~~disclosure.