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# Establishment of a Gaseous pH Control Concept in Microbioreactors

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**Abstract.** Existing methods for pH control in bench-scale bioreactor systems often cannot be directly adapted for microbioreactors. This is because microbioreactors are commonly designed to work with constant volumes, operate bubble-free and have no headspace, which technically rules out any possibility of adding acid/base solution for pH control in microbioreactors. This work reports on the establishment of a gaseous pH control concept in microbioreactors where pH control was achieved by dosing of ammonia ($NH_3$, 20 000 ppm) and pure carbon dioxide ($CO_2$) gases to respectively; increase and lower the pH of the reactor content. It encompasses the establishment of an optical pH measurement by means of a fluorescent sensor spot, realization of the necessary gas connections, mixing of gases, and gas-exchange via a thin semi-permeable poly(dimethylsiloxane) (PDMS) membrane. It was shown that addition of $NH_3$ and $CO_2$ gases coupled to a simple on/off controller results in a satisfactorily control performance (pH control accuracy = $\pm$ 0.1 of the set point value and system responses of a few minutes were achieved) within the dynamic measuring range of the optical sensor spot which is between pH 6 and 8.

**Keywords:** microbioreactor, optical pH sensor, pH control, feedback controller

## 1. Introduction

Microbioreactors are miniaturized bioreactor systems (typically with working volume less than 1 mL) developed specifically to facilitate high throughput and low cost bioprocessing under well-controlled conditions (e.g. fermentation experiments). Advantages of microbioreactor system include flexibility to run the microbioreactor in either batch, fed-batch or continuous (e.g. chemostat) operation, good mass and heat transfer rate and have the capacity to acquire real-time experimental data which increases the amount of information gained per experiment. Additionally, due to their small size, microbioreactors also offer a number of cost reducing advantages for studying biological processes. These include significant reductions in running cost per experiment (low substrate and utilities consumption, low waste generation), less space required for parallel operation and the possibility of making the microbioreactor disposable to minimize reactor preparation efforts [1].

Whilst methods to control reactor variables, namely temperature and dissolved oxygen concentration, do exist [2-5], suitable methods to control pH in microbioreactors are still in the development phase. pH has a strong impact on the cell growth and production rate and is important in all enzyme-catalyzed processes as well. In fermentation processes, conversion of carbon sources typically results in a generation of acid and carbon dioxide. This increases the amount of protons ($H^+$) and thus, acidifies the reactor's content. Consequently, if pH is not controlled at its set point value, culture pH will decrease over time and may halt the cell growth rate due to the fact that the reactor pH shifts away from ideal culture conditions as a consequence of metabolic activity. As for enzyme-catalyzed processes, it is of utmost importance to keep the pH of the reactor close to its optimum value to avoid any production of unwanted product or inactivation of the enzyme. This is because enzymes have a very narrow optimal pH band where they work at their optimal

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rate. Given the importance of this variable, a tight pH control of the microbioreactor contents is indeed crucial for a successful microbioreactor experiment.

Currently, there are number of methods available for pH control in a microbioreactor [1]. This includes by the use of a buffer [2], intermittently injecting base or acid [3,5] and by addition of gas (e.g. ammonia, $NH_3$ and carbon dioxide, $CO_2$) through a semi-permeable poly(dimethylsiloxane) (PDMS) membrane [4,6]. Table 1 summarizes advantages and disadvantages of each method. In this work, we further develop the gaseous pH control concept for microbioreactors that was first introduced by Maharbiz *et al.* [4] and De Jong [6]. We propose the addition of either a gas mixture consisting of nitrogen gas containing 20000 ppm of ammonia or carbon dioxide gas, coupled with a simple on/off controller for pH control in microbioreactors. Introduction of these gases in the reactor was achieved through a thin semi-permeable membrane, thus keeping the microbioreactor volume constant. It is first described in detail how this gaseous pH control concept was achieved by means of a closed-loop circuit consisting of an optical pH sensor, the necessary gas connections, valves, and the on/off controller. The controller performance is then evaluated in terms of control accuracy, response time and set point tracking capability.

Table 1 Various methods for pH sensing and control in microbioreactors.

| Source | pH sensor | Measurement approach | pH regulation | Pros | Cons |
|---|---|---|---|---|---|
| [2] | pH optode | *in situ* | Buffered system | (1) Simple<br>(2) Easy handling | (1) Limited buffer capacity<br>(2) Scale-up: not feasible |
| [3,5] | pH optode | *in situ* | Acid/base addition | (1) pH can be maintained longer<br>(2) Scale-up: feasible | (1) Limited reactor volume<br>(2) Concentrated acid/base leads to local high/low pH |
| [4,6] | pH ISFET | *in situ* | Gas diffusion through PDMS membrane | (1) Not limited by reactor volume<br>(2) Scale-up: feasible | (1) Complicated fluidics for gas supply |
| [This work] | pH optode | *in situ* | | | |

# 2. Materials and Methods

## 2.1. Microbioreactor design and fabrication

A disc-shaped microbioreactor design with an operating volume of 100 µL was used for the characterisation of the pH control system by conduction of titration and set point tracking experiments. The reactor was completely made of a poly(dimethylsiloxane) (PDMS) and fabricated through a simple micromachining processes (i.e. involving few casting and curing steps). The microbioreactor used was equipped with necessary measurements and control system to support a typical aerobic fermentation processes. Programs for measurement and control routines were written in LabVIEW v8.5 software (National Instruments, Austin, TX, USA), and were implemented by interfacing LabVIEW with a data acquisition (DAQ) card (NI USB-6229, National Instruments) for data logging and sending signals to actuators. Details for the design and fabrication strategies of the microbioreactor prototype have been described elsewhere [7].

## 2.2. Gaseous pH control scheme

### 2.2.1. pH measurement

pH was measured by means of an optical measurement with the use of a fluorescent sensor spot. In every measurement, a sine-modulated light (44 kHz) from a blue LED (465 nm, NSPB500S, Nichia Corporation, Tokushima, Japan) was shone onto an optical sensor spot which was mounted on the inside of the reactor. This sensor spot in turn emitted fluorescent light at 520 nm peak wavelength, with the same frequency as the

incoming light, but with a phase lag. The emitted light was then collected with a silicon detector (Thorlabs PDA36A, Thorlabs Inc., NJ, USA) and the resulting voltage was read by LabVIEW. A lock-in amplifier programmed in LabVIEW then measured the phase shift between the outgoing and the incoming signal, and this shift then translated directly to pH values. The optical pH sensor used has a measurement accuracy of about 0.01 pH units with a response time ($t_{90}$) of less than 90 s [1]. From our calibration data (data has been shown in [7]) the dynamic measurement range of the sensor spot is ranging between pH 5.5 to 8. Within this range, the sensor response is almost linear with a sensitivity of about $10^{o}$ phase angle per pH unit.

### 2.2.2. pH control algorithm

The pH of the reactor content was controlled by an on/off controller. The on/off controller was implemented in LabView™ v8.5 software (National Instruments Corporation, TX, USA), and interfaced with the reactor hardware using A/D cards (USB-6229 and PCI-4461, National Instruments Corporation, TX, USA). The operation of the pH control algorithm is schematically presented in Fig. 1.

First, the LabView program will compute the deviation (error) between the desired set point value, $pH_{sp}$ and the measured value, $pH_m$. The controller was made to include a tolerance limit (dead band) around the set point. The dead band prevents the dosing valves from rapidly or continuously switching because the measured value never exactly fits the set point value when using an on/off controller in this type of systems. During pH control, no titration will take place if the pH deviation from the set point is within the tolerance limits. If the error is larger than the tolerance limit and positive ($pH_{sp} > pH_m$), $NH_3$ gas will be added and if the error is negative ($pH_{sp} < pH_m$), $CO_2$ gas will be added. A latching relay was used to control the state ('open' or remain 'closed') of the solenoid valve for gas addition. During addition of gas, the controller will first depressurize the gas connection (depressurizing period was set for 1 s) before opening up the dosing valve. Other settings to be adjusted by the user are the gas bottle pressure (or the inflow gas flow rate via the mass flow controller) which determines the amount of gas diffusing through the membrane, the tolerance limits (dead band), and of course the pH set point. In this application, the on/off controller was tuned to give the smallest step change possible in both acidic and basic directions.

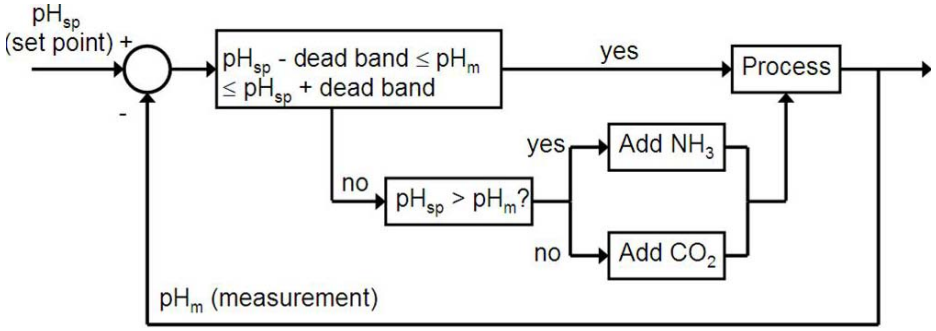


Fig. 1. Block diagram of the on/off pH controller.

### 2.2.3. Gas connections

pH control was accomplished by dosing of either a gas mixture containing 20 000 ppm ammonia gas, $NH_3$ (the rest is nitrogen gas) or pure carbon dioxide gas, $CO_2$ respectively to increase and decrease the pH of the reactor content. The gas connections for the pH control are illustrated in Fig. 2. Both gases were supplied from pressurized gas bottles equipped with two-stage gas regulators. The gas flow from the gas bottles was quantified by using mass flow meters (SHO-RATE, Brooks Instrument, Holland, Model 504-ES-22-F2B). For gas addition, 2-way Polyetheretherketone (PEEK) solenoid valves (Bio-Chem Valve, Cambridge, UK, 038T2B12-32-5) were connected directly to each of the mass flow controllers (one for $NH_3$ and one for $CO_2$). Both valves were connected in parallel and remain closed unless a voltage supply is connected. An additional solenoid valve each was installed to depressurize the gas line prior to dosing of gas into the reactor. This additional valve is necessary because the continuous gas flow from the gas bottle causes the pressure in the tubing to increase when the solenoid valves are in the 'closed-position', until the whole tubing has the pressure which was adjusted at the regulator. Overpressure in the gas connections is not desirable because it may lead to an undesired overshoot in reactor pH. The $NH_3$ and $CO_2$ gas lines were then joined together via a T-connector and introduced into the microbioreactor by connecting the gas line, $F_1$ to

the top layer of the microbioreactor. During pH control, only one gas was dosed at a time. Part of the gas diffuses through the semi-permeable membrane and induces the desired pH changes. The remaining gas flows out through the outlet port, $F_2$ and is removed with the ventilation. All gas connections were established by standard Perfluoroalkoxy (PFA) tubing with an outer diameter of 3.175 mm using fittings from Upchurch Scientific.

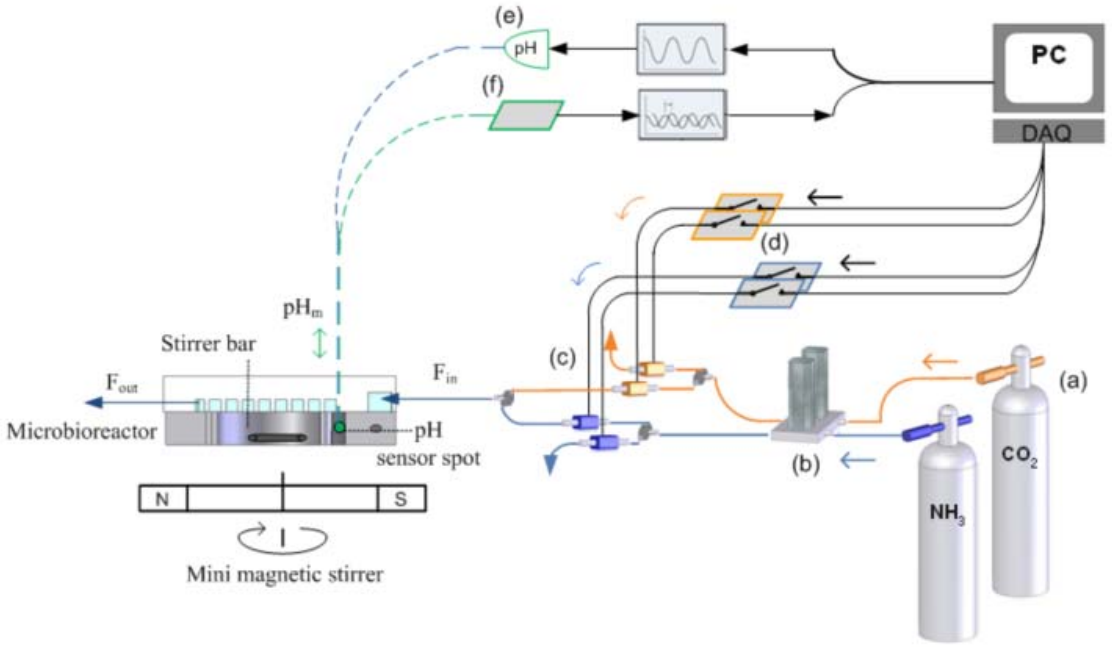


Fig. 2. Schematic of the gas connections for the gaseous pH control scheme. (a) $NH_3$ and $CO_2$ gas bottles (b) mass flow meters (c) solenoid valves (d) relays (e) LED (465 nm) (f) photo detector. Dashed lines (--), represent optical fibers, black solid lines represent electrical wiring (-), and blue/orange solid lines represent tubing for fluidics (-/-).

## 3. Results and Discussions

Prior to tuning of the on/off controller, the system behavior was first evaluated by conducting two different sets of titration experiments (Fig. 3). In the first titration experiment, $CO_2$ gas was dosed into a solution with a starting pH of 8 at a constant dosing pulse length of 1 s until the solution reached a final pH of about 6. In the second titration experiment, a reverse titration was made in which $NH_3$ gas was dosed into the reactor (initial pH of the solution is 6) at a constant dosing pulse length of 1 s until the solution pH reached an end value of about pH 8.2.

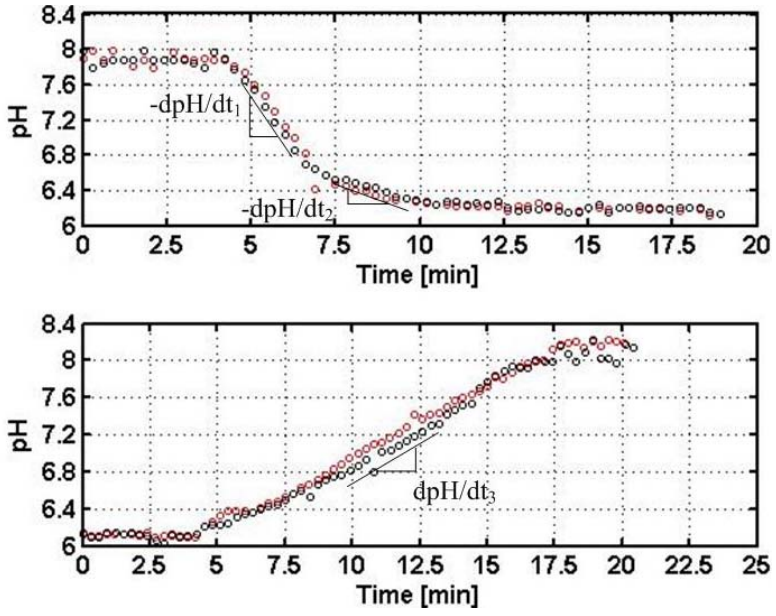

Fig. 3. Responses from the titration experiments. Top: YPD medium ($pH_o$ = 8) titrated with $CO_2$ gas (at 2 bar and 25°C) to a final pH of about 6.3. Bottom: YPD medium ($pH_o$ = 6) titrated with $NH_3$ (20 000 ppm) – $N_2$ gas mixture (at 1 bar and 25°C) to a final pH of about 8.2.

For both experiments, tolerance limits of the controller were set to $\pm$ 0.1 of $pH_{sp}$, and the pressure of $NH_3$ and $CO_2$ gas bottles was regulated to 1 bar and 2 bar, respectively. The limited viable measuring range of the pH sensor spot does not permit to perform experiments beyond pH 6 and 8 [1]. The titration experiments were conducted in YPD medium (a common rich medium for *S. cerevisiae* cultivation [1]) at room temperature ($T_m$~25°C). By performing the experiment in YPD medium instead of distilled water, a more realistic and stable pH response from the process can be obtained due to the mild buffering capacity offered by the medium.

From the response obtained, it was seen that upon dosing of $CO_2$ gas; the pH of the reactor content drops significantly at a very fast rate (indicated by a steeper slope of the curve, $-dpH/dt_1$) until the solution pH reaches a pH value of about 6.5. From this point onward, the solution pH decreases slowly (indicated by the second slope of the curve, $-dpH/dt_2$) until it reaches a final pH value of about 6.2 to 6.3. This behavior is explained from the acid-base equilibrium of the system and the reactions that took place when $CO_2$ gas dissolved in the water. When $CO_2$ dissolves in water, it instantly reacts with $OH^-$ ions from the water to produce hydrogen ions, $H^+$ and forms carbonic acid, $H_2CO_3$ which then rapidly dissociates into bicarbonate, $HCO_3^-$ ($pKa_1$ at 25°C = 6.36) and also into carbonate, $CO_3^{2-}$ ($pKa_2$ at 25°C = 10.32) [8]. This lowers the solution pH (pH = -log [$H^+$]). In the mild basic region of pH 7.5 – 8; due to the excess of hydroxyl ions, $OH^-$, and since the solution is not buffered (pH is nowhere near $CO_2$ pKa values), a sharp reduction in solution pH upon the addition of $CO_2$ was observed. However, in the slightly acidic region (~ pH 6 - 6.5), the solution pH is very close to the pKa of the first dissociation of $H_2CO_3$ ($pKa_1$ at 25°C = 6.36). Around this pH the solution will be buffered and thus the rate of change of the pH decreases. On the contrary, $NH_3$ gas undergoes a much more straightforward reaction when it dissolves in water. It will react with the hydrogen ($H^+$) ions from the water forming ammonium ions, $NH_4^+$ and hydroxyl ions ($OH^-$) [8]. Further addition of $NH_3$ gas will consume more hydrogen ($H^+$) ions and thus, increase pH of the solution. Also, during the whole experiment, the working pH range (pH 6 to pH 8) was far away from the dissociation constant of ammonia ($pKa_{NH3}$ at 25°C = 9.25). This explains why the increase of solution pH following the dosage of $NH_3$ gas into the reactor only has one slope, $dpH/dt_3$. Based on the results of the titration experiment, the on/off controller was tuned to induce pH changes that are as small as possible and then, a set point tracking experiment was performed to evaluate the controller performance in terms of its accuracy and response time. Result obtained from the set point tracking experiment is presented in Fig. 4.

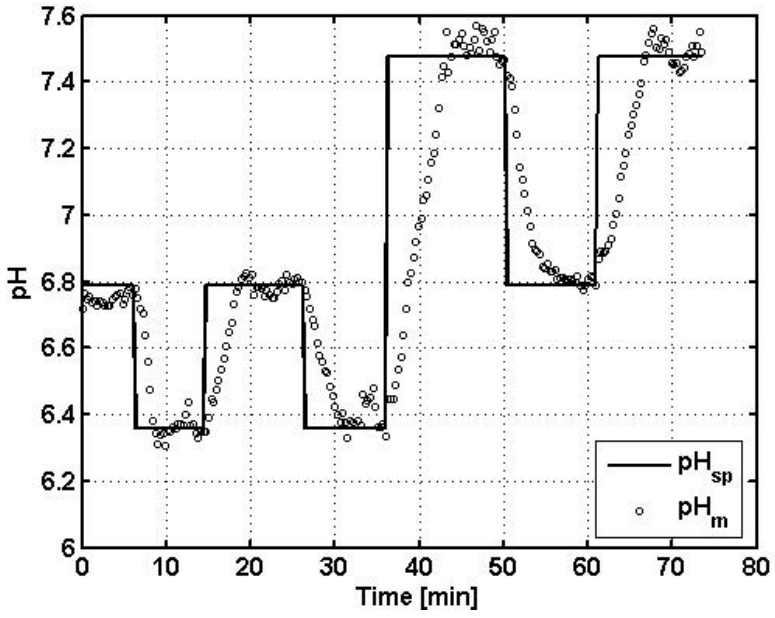

Fig. 4. Closed loop response of the on/off pH controller for different set point values ($pH_{sp}$ = 6.8, 6.3 and 7.5), starting from pH 6.8.

The results of the set point tracking experiment (Fig. 4) demonstrate that the pH on/off controller has a fast set point tracking capability for a series of downward (pH 6.8 to pH 6.3) and upward (pH 6.8 to pH 7.5) step changes in the pH set point. In this application, the dosing pulse lengths of both gases were adjusted such as to induce pH changes that are as small as possible. This has the drawback that it may prolong the time needed to achieve the desired set point, especially if the pH set point is changed significantly. However, small step changes ensure a more stable operation in the sense that a large pH fluctuation around the set point due to dosing of gas for pH control can be avoided. Also, in cultivations of e.g. yeast or in enzymatic reactions the pH seldom changes very quickly, and thus a slower but more precise control is to be preferred. The results of the set point experiment also show that by only adjusting the gas dosing pulse length accordingly, controller accuracy as high as $\pm$ 0.1 pH units around the $pH_{sp}$ can be achieved. The largest pH step change made was from a pH set point of 6.3 to a pH set point of 7.5, and in this case the rising time was approximately 6.5 minutes. During the pH step changes, only a short delay (less than a minute) was recorded. This is probably due to a high gas transport rate through the PDMS membrane and good mixing via the micro-impeller incorporated in the reactor which together allow for a fast response [7]. The responses obtained from the pH feedback control loop are comparable to the responses produced from a gaseous pH control system developed by De Jong [6] where a system delay time of approximately 60 seconds and a rising time of a few minutes have been recorded. In the current setup used for testing, adjustment of the dosing pulse lengths has to be done manually. However, optimal settings for the pulse length can be programmed in the software controlling the setup, such that the settings, and by this the controller gain, are changed automatically when the pH set point value is altered.

## 4. Conclusion

A gaseous pH control method was developed and was demonstrated to provide satisfactory pH control performance in the microbioreactor system. The proposed method is very user-friendly and in depth knowledge of the microbioreactor system is not necessary to operate the pH controller. The necessary apparatus (mass flow meters, solenoid valves, and relays) to transport the gas into the microbioreactor and its basic functionality has been presented. It was shown that addition of $NH_3$ and $CO_2$ gases coupled to a simple on/off controller results in a satisfactorily pH control performance (pH control accuracy = $\pm$ 0.1 of the set point value and system response times of a few minutes were achieved) within the dynamic measuring range of the optical sensor spot which is between pH 6 to 8.

## 5. Acknowledgements

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