Review of Critical Ranges in pH Control for Bioprocesses

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ABSTRACT

This paper presents an extensive review of the importance of pH in bioprocesses gathered from various literatures in the past few years. It has been found in the review that controlling pH in bioprocesses are not limited in providing optimum growth conditions to the organisms, but also in neutralizing excess reagents, acidic by-products and obtaining high yield of selective by-product. It was also found that high yield of product, for certain reaction and kinetic, can be achieved in semibatch operation rather than in CSTR or batch operations.

Keywords: *pH control; bioprocess; fermentation; aerobic-anaerobic; nitrification-denitrification.*

1 INTRODUCTION

The importance of pH control in various industries such as baking, brewing, canning, chemicals, cleaners, dyes, electroplating, fermentation, pharmaceuticals, pigments, pulp and paper, sewage, textiles and water treatment have been briefly noted by McMillan [1]. A more concise information on the optimum conditions, such as temperature, pH and reactor's size, in treating wastewater at various stages and processes had been prepared by Corbitt [2].

In bioprocesses, microorganisms are part of the chemistry, and they are susceptible to changes in the microorganism's living environment (medium) such as temperature, dissolved oxygen and pH. This paper intent to present an extensive review on the importance of pH and its control strategies in bioprocesses (i.e. aerobic-anaerobic processes and nitrification-denitrification processes) gathered from various literatures in the past few years.

2 NITRIFICATION-DENITRIFICATION PROCESSES

In the nitrification process, ammonium (NH_4^+) is reduced in two steps. The first reduction is into nitrite (NO_2^-), while the second reduction is into nitrate (NO_3^-) as shown in Eq. 1 and 2 below [3].

$$2\,NH_4^+ + 3O_2 \xrightarrow{Nitrosomonas} 2\,NO_2^- + 2\,H_2O + 4\,H^+ \quad (1)$$

$$2\,NO_2^- + O_2 \xrightarrow{Nitrobacter} 2\,NO_3^- \quad (2)$$

The nitrification process requires aeration for the organisms, *Nitrosomonas* and *Nitrobacter*, to live. The nitrate is further reduced to nitrogen gas through denitrification process as shown in Eq. 3 [4] and Eq. 4 [5].

$$2\,NO_3^- + org.\ matter \xrightarrow{bac.} N_2 + CO_2 + H_2O \quad (3)$$

$$6NO_2^- + 3CH_3OH \xrightarrow{bac.} 3N_2 + 3CO_2 + 3H_2O + 6OH^- \quad (4)$$

Among the organisms which are capable of reducing NO_3^- into N_2 are *Achromobacter, Aerobacter, Bacillus, Micrococcus*, etc. The organisms for denitrification process do not require aeration to live. Some of the latter studies in nitrification and denitrification, which involved pH control, are illustrated below.

Van Kempen et al. [6] and Hellinga et al. [7] had elaborated a new-patented method in the removal of nitrogen from an ammonium-rich wastewater. In this method, called SHARON®, the ammonium is nitrified and denitrified between pH 6.8 and 8.0 at high temperature in a single reactor without the need of sludge retention. The nitrification and denitrification are separated by switching the aeration on and off. Due to high ammonium concentration and high reaction rates, pH control becomes a key parameter in driving the nitrification and denitrification processes. During the nitrification process, NaOH solution is pumped to the reactor to within the desired control range. At the end of the nitrification process, the aeration is shut off and the denitrification begins. During the denitrification process, methanol replaces the NaOH solution as the pH control element and also acts as a carbon source. By controlling the pH to within the specified range, a 90% removal of nitrogen can be achieved.

Cecen et al. [8] studied the effect of pH control in the nitrification of a high-strength fertilizer wastewater. The studies had been conducted in a 3-liter and 5-liter reactors of batch activated sludge and CSTR systems, respectively, using *Nitrosomonas* and *Nitrobacter* microorganism. The reactors had been operated and controlled at several selected set points (pH). They had found that the combined effect of high ammonia and pH greater than 8.5 would inhibit Nitrobacter. For both reactor systems, a pH range between 6.5 and 8.5 was the optimum condition for the microorganism activity.

Beaubien et al. [9] had developed a control and regulation method based upon gas production rate measurements to monitor the metabolic activity in the denitrification of ground water supply. The studies were conducted in an 8-liter reactor of fed-batch system. The set points (pH) were varied from time to time. They had found that changing the set points have profound effect on the microorganism metabolic activity, which led to a variation in gas production rate. A high metabolic activity (80 to 100%) was observed when the pH is between 6.5 and 8.5; while, a sharp drop in metabolic activity (0 to 30%) was seen when the pH is less than 6.5 or the pH is greater than 8.5.

3 AEROBIC -ANAEROBIC DIGESTIONS

In general, aerobic process can be regarded as a process whereby the microorganisms require oxygen to live, while in anaerobic process, the microorganisms does not require oxygen to live. These two processes are important largely in fermentation and wastewater treatment industries as will be elaborated here.

Horiuchi et al. [10] studied the production of organic acids such as butyric acid, acetic acid and propionic acid from organic wastewater by anaerobic acidogenic bacteria in a 2-liter fed-batch fermentation reactor. The effect of pH control to the yields of butyric, acetic and propionic acids was studied. The pH in the fermentor was kept constant at pH 5, 6, 7 and 8 by pumping 5 M H_2SO_4 or 5 M NaOH through out the experiment. They had found that the yield of each organic acids was depended on the pH of the medium. Butyric acid and acetic acid were predominantly produced between pH 5 and 7; while, acetic acid and propionic acid were predominantly produced at pH 8. They had attributed the differences in yields to the different types of bacteria present in the medium, which were active only at certain range of pH.

In the production of polysialic acid from N-acetylneuraminic acid by *Escherichia coli*, Zhan et al. [11] had found that the highest yield of polysialic acid can be obtained at pH 6.4 in a 15-liter fermentor of fed-batch system. The pH in the fermentor was kept constant by pumping 30% NaOH solution invoked by a pH controller. They also found that the yield of polysialic acid was doubled in a fed-batch system compared to a batch system, where the carbon and nitrogen sources were continuously fed-in to the system.

Liu et al. [12] had studied the production of ethanol by *Zymomonas mobilis* in a 5-liter reactor of fed batch system. The optimum pH for *Z. mobilis* activity, which resulted in high production rate of ethanol, was at 5, and an on-off control strategy had been written in software in order to maintain the pH in the fermentor. It was done by pumping one ml of HCl solution when ÄpH was positive or one ml of NaOH solution when ÄpH was negative using peristaltic pumps for every 10 s.

Weuster-Botz et al. [13] conducted their studies on the production of GDP-mannPP by *Escherichia coli* in 100-ml shaking flasks of fed-batch system. A computer had been used to monitor and control the intermittent feeding of substrates by tracking the predefined feeding profiles. They had found that by keeping the medium stabled at pH 7, higher cellular activities were recorded which resulted in high yield of GDP-mannPP. They had also observed a higher production of GDP-mannPP in fed-batch system compared to conventional batch system. In their study, a PID control strategy was employed to regulate a 10% NH_4OH solution into the flasks via a piston pump in order to maintain the setpoint.

Fu et al. [14] had studied a batch fermentation of lactic acid from lactose by *Lactobacillus plantarum* in a two liter stirred tank reactor. The optimum pH for cell growth

and lactic acid production was found between 5 and 6, while a pH of less than 4 would inhibit cell growth. In another experiment without pH control, the accumulation of lactic acid reduced the pH, which eventually inhibited the growth of *L. plantarum*. A pH controller had been used to regulate a peristaltic pump, which delivered 12 N NaOH solution, in order to maintain the desired control range.

O'Donnell et al. [15] made a studies on the effect of pH control to the production of recombinant protein using *Aspergillus niger*. The studies had been carried out in a 15-liter bioreactor system. They had found that the optimal growth for *A. niger* was at pH 3 with maximum protease secretion. But, the yield of recombinant protein was minimal. Consequently, they had maintained the pH in the reactor at the lowest baterial activity i.e. pH 6 which resulted in a 10-fold more recombinant protein being produced.

Dilsen et al. [16] conducted a study on the growth of *Staphylococcus carnosus* for the production of peptide. The study had been conducted in a 200-ml batch bubble column reactor equipped with pH control systems that include pH sensor, pH controller and dosing pump. The pH of the medium had been maintained at pH 4, 5, 6, 7, 8 and 9 by dosing 4 M NaOH solution. They had found that a pH of between 5 and 8 had recorded high growth rates of *S. carnosus*; while, a pH of less than 4 or above than 9 had recorded almost negligible growth rate. A batch study without pH control had also been conducted, and the result showed that the pH in the medium had declined to 4.5 not long after the test started. This pH-drop was caused by the "unwanted" production of organic acids (such as acetic acid and lactic acid) which in turn inhibited the growth of *S. carnosus*.

In a pilot scale study of rapamycin production by *Streptomyces hygroscopicus*, Chen et al. [17] had found that a higher biomass concentration and higher rapamycin production were obtained when the medium was controlled between pH 6.2 and 6.8. The study was conducted in a 100-liter reactor of fed-batch system. A supervisory computer was used to monitor and control the stirrer speed, aeration rate, vessel pressure, pumps' activation and the culture temperature. A peristaltic pump which supplied 10% NaOH solution was activated when the pH dropped below 6.2, while another peristaltic pump would be activated when the pH surpassed pH 6.8.

Ghaly [18] conducted a study on anaerobic digestion of dairy manure in a 155 liters of a two-stage unmixed reactor system. The pH in the reactor was monitored by a pH controller and kept constant between 5.7 and 6.0 by pumping NaOH via peristaltic pump. The NaOH pump was only activated when the pH dropped below 5.7. Ghaly had found that by controlling the pH between 5.7 and 6.0, the biogas production rate increased, while the COD and solid mass concentration had reduced by a factor of three compared to a reactor system that had no pH control. In the experiment without pH control, the accumulation of organic acids resulted in a pH-drop to 3.3, which in turn, inhibited the microorganisms and lowered the biogas production rate.

Wittmann et al. [19] studied the inhibitory effect of ammonium on the growth of polychlorinated xenobiotic-degrading bacterium *Mycobacterium chlorophenolicum* in a 3.5-liter reactor of fed-batch system. They had devised a pH control strategy that was based on the consumption rate of NH_4OH and the rate of pH decline in order to stabilized the pH at the optimum growth of the bacteria i.e. pH 7. The pH control strategy was translated into a computer software. The additions of 0.33 M H_3PO_4 reagent and 2 M NH_4OH solution as the nitrogen source and neutralizing agent were controlled by a computer, based on the devised pH control strategy. With this strategy, a faster growth rate of *M. chlorophenolicum* was acheived compared to conventional batch system. Further more, the yield of biomass was doubled in fed-batch system compared to the batch system.

4 CONCLUDING REMARK

The application of pH control in various bioprocesses has been presented. Based on the reviews presented, it can be concluded that pH control is needed to,

a. neutralize excess reagent such as acid or base in order to maintain the pH within setpoint,
b. neutralize unwanted by-products such as H^+, OH^- or organic acids,
c. provide optimum growth for the micro-organisms,
d. and obtain high yield of by-products by reducing the growth rate of microorganisms.

It is also noted from these literature that the product's yield for certain reaction and kinetic is higher in fed-batch system than in CSTR and batch systems. The high yield in fed-batch is attributed to the continuous supply of nutrients which may prolong the growth of the microorganisms and, in certain cases, the neutralization of organic acids which without the neutralization may accumulate and inhibit the microorganisms. A summary of the applications and significants of pH control has been summarized in Table 1.

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Table 1 – Summary of the applications and significants of pH control in bioprocesses

Processes	**Control range (pH)**	**Reactors (Size)**	**Control objective**	**References**
Prod. of polysialic acid	6.4	Fed-batch (15 L)	High yield	Zhan et al. (2002)
Prod. of organic acids	5 – 7 (butyric, acetic)	Fed-batch (2 L)	Opt. condition, high yield	Horiuchi et al. (2002)
	8 (acetic, propionic)			
Prod. of ethanol	5 ± 0.05	Fed-batch (2 L)	opt. condition	Liu et al. (2001)
Prod. of GDP-mannPP	7.0	Fed-batch (0.1 L)	High yield	Weuster-Botz et al. (2001)
Prod. of recombinant protein	6	Batch	High yield	O'Donnell et al. (2001)
Prod. of peptide	5 – 8	Batch bubble column	High yield	Dilsen et al. (2001)
Prod. of lactic acid	5 - 6	Batch (2 L)	opt. cell growth, high prod.	Fu et al. (1999)
Prod. of rapamycin	6.2 – 6.8	Fed-batch (100 L)	High biomass conc., high yield	Chen et al. (1999)
Prod. biogas, solid reduction	5.7 – 6.0	Two-stage reactor (155 L)	Opt. condition, high production	Ghaly (1996)
Prod. of biomass	7.00 ± 0.05	Fed-batch	High biomass conc.	Wittmann et al. (1995)
Removal of nitrates	6.5 – 8.5	Batch (3 L), CSTR (5 L)	Opt. condition	Cecen et al. (1995)
Removal of nitrates, nitrites	6.8 – 8.0	CSTR	Opt. condition, high removal	Hellinga et al. (1998)
				Van Kempen et al. (2001)
Removal of nitrites	6.5 – 8.5	Fed-batch (8 L)	Opt. condition	Beaubien et al. (1995)