Kaunas University of Technology

Faculty of Electrical and Electronics Engineering

Model-Free Adaptive Control of pH in Fed-Batch Biotechnological Process

Master's Final Degree Project

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Kaunas, 2026

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Control technologies (6211EX014)

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Declaration of Academic Integrity

I confirm that the final project of mine, Muhammad Suleman Jamal, on the topic ‘‘pH Control of a Fed-Batch Biotechnological Process Using Model Free Adaptive (MFA) Controller’’ is written completely by myself; all the provided data and research results are correct and have been obtained honestly. None of the parts of this thesis have been plagiarised from any printed, Internet-based or otherwise recorded sources. All direct and indirect quotations from external resources are indicated in the list of references. No monetary funds (unless required by Law) have been paid to anyone for any contribution to this project.

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SUMMARY

In this thesis, the problem of pH regulation in fed batch biotechnological processes is addressed, where the process dynamics are nonlinear, time variant, and highly uncertain. The modeling of such systems is often complex, and the usual model-based control techniques are not very effective. The goal of this work was to design and test a model-free adaptive control strategy to keep the pH at the desired pH without the use of an explicit mathematical model of the process.

Based on literature, a fed-batch process model was implemented in MATLAB/Simulink and firstly, a classical fixed PI controller and adaptive gain scheduling PI controller was implemented as a reference approach. Later, an adaptive (model-free) controller (MFA) was designed that modifies the control actions as per the process of input-output data. Each one of the three controllers were tested under various conditions, with a sampling time of 1s.

The simulation results show that the model-free adaptive controller achieves improved performance in pH regulation compared to the classical fixed PI controller and adaptive gain scheduling PI controller, particularly in terms of tracking accuracy, and adaptability to disturbances and modeling uncertainties. The MFA approach has proven to be effective in achieving stable pH control without the need to know the system dynamics accurately.

The greatest value of this work is the proof of an effective model-free control strategy for fed-batch bioprocesses for pH regulation. It can be used in systems where the modeling approach is complicated or plants with a lower amount of available data. Future studies can be done for experimental validation of the designed MFA controller and optimization of the control strategy for practical applications in different engineering applications.

Muhammad Suleman Jamal. Adaptyvus pH valdymas netaikant modelio, skirtas periodiniam su pamaitinimu biotechnologiniam procesui. Magistro baigiamasis projektas / vadovas prof. Vytautas Galvanauskas; Kauno technologijos universiteto Elektros ir elektronikos fakultetas.

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SANTRAUKA

Šiame darbe nagrinėjama pH reguliavimo problema periodiniuose su pamaitinimu biotechnologiniuose procesuose, kuriose proceso dinamika yra netiesinė, kinta laike ir yra labai neapibrėžta. Tokių sistemų modeliavimas dažnai yra sudėtingas, o įprasti modeliais pagrįsti valdymo metodai nėra labai efektyvūs. Šio darbo tikslas buvo sukurti ir išbandyti adaptyvią valdymo strategiją be modelio, kad pH būtų palaikomas norimame pH lygyje nenaudojant aiškaus matematinio proceso modelio.

Remiantis literatūra, MATLAB/Simulink buvo įdiegtas periodinio su pamaitinimu proceso modelis, o pirmiausia klasikinis fiksuotas PI reguliatorius ir adaptyvus stiprinimo numatymo PI reguliatorius buvo įdiegtas kaip etaloninis metodas. Vėliau buvo sukurtas adaptyvus (nenaudojantis modelio) reguliatorius (MFA), kuris modifikuoja valdymo veiksmus pagal įvesties-išvesties duomenų procesą. Kiekvienas iš trijų valdiklių buvo išbandytas įvairiomis sąlygomis, o grįžtamojo ryšio signal intervalas buvo 1 s.

Modeliavimo rezultatai rodo, kad adaptyvus reguliatorius be modelio pasiekia geresnę pH reguliavimo kokybę, palyginti su klasikiniu fiksuotu PI reguliatoriumi ir adaptyviu stiprinimo numatymo PI reguliatoriumi, ypač sekimo tikslumo ir trikdžių kompensavimo bei modeliavimo neapibrėžtumų požiūriu. MFA metodas pasirodė esąs veiksmingas siekiant stabiliaus pH valdymo, jam nereikia tiksliai žinoti sistemos dinamikos.

Didžiausia šio darbo vertė yra veiksmingos valdymo be modelio strategijos įrodymas periodinių su pamaitinimu bioprocesų pH reguliavimui. Jis gali būti naudojamas sistemose, kuriose modeliavimas yra sudėtingas, arba gamyklose, kuriose yra mažesnis turimų duomenų kiekis. Ateityje gali būti atliekami eksperimentiniai suprojektuoto MFA reguliatoriaus patvirtinimo tyrimai ir valdymo strategijos optimizavimas praktiniam pritaikymui įvairiose inžinerinėse programose.

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List of Abbreviation

PI	Proportional-Integral
MFA	Model-Free Adaptive
IAE	Integral of Absolute Error
RMSE	Root Mean Square Error
pH	Potential of Hydrogen
X	Biomass concentration
S	Substrate concentration
P	Product concentration
t	Time
μ	Specific growth rate
KS	Half-saturation constant
KI	Substrate inhibition constant
Pd	Product inhibition constant
I	Product inhibition exponent
α	Growth-associated product formation coefficient
β	Non-growth associated product formation coefficient
FpH	Alkali flow rate
OUR	Oxygen uptake rate
MPC	Model Predictive Control
ANN	Artificial Neural Network

Introduction

Biotechnological processes are increasingly used in the modern industry in the production of pharmaceuticals, enzymes, biofuels and other biochemical products of high added value. These processes are normally performed in bioreactors under well controlled environmental conditions. Among the most important variables that need to be controlled in cultivation, pH is of great relevance, as it directly affects the growth of microorganisms, metabolic pathways, enzyme activity and the formation of products.

In fed batch biotechnological processes, the pH control is a particularly difficult task. The process dynamics are nonlinear, time dependent and subject to large disturbances. Continuous biomass growth causes a variation in the metabolic activity, which causes fluctuating rates of hydrogen ion production or consumption during cultivation [1]. In addition, the titration properties of the cultivation medium are very nonlinear, so minor deviations of the alkali's addition may lead to significant deviations in pH especially near neutrality [2]. These properties make it considerably difficult to design and implement high performance pH control systems.

Despite these difficulties, classical proportional integral (PI) controllers are still very common in industrial practice because of their simplicity and ease of implementation [3]. However, fixed parameter PI controllers are often not good on processes with strong nonlinearities and time varying behaviour [2,3]. To overcome these limitations, adaptive strategies have been proposed; in particular gain scheduled adaptive PI that adjust parameters online using process related variables such as the oxygen uptake rate (OUR) [1].

Although gain scheduled adaptive PI can improve the system performance, such methods are still based on explicit process models and predefined adaptation rules [1]. In the case of complex biotechnological systems, it is difficult to obtain good models, in addition to the validity of the obtained models in wide operating ranges, which motivates Model Free Adaptive (MFA) control, which aims at high quality control without the use of explicit process models [2,3].

The overall objective of this thesis is to investigate the pH control in a biotechnological process with the use of an adaptive MFA controller [2]. Within this framework, the present report deals with implementing the biotechnological process model in the MATLAB Simulink and conducting preliminary controller studies with special emphasis on the MFA control.

Research Aim:

This work is aimed at designing and testing a model-free adaptive (MFA) controller for the pH regulation of a fed-batch biotechnological process to ensure accurate performance without the need for developing an explicit mathematical model of the process.

Research Objectives:

1. To develop a non-linear fed batch biotechnological process model using the MATLAB Simulink environment.
2. To validate the implemented model by using simulation under the representative operating condition;
3. In order to implement baseline controllers, a conventional PI controller and an adaptive PI controller adopted from the literature.
4. To develop an MFA controller and test the control performance.

It must be stressed that the PI controller is implemented strictly on the original formulation presented in [1] and is only used as a benchmark for comparison. The main focus of this work is thus in the implementation of biotechnological process given in [1] and MFA controller.

1. Bioreactors

A bioreactor is a special tank which is used to grow living cells like bacteria, yeast, fungi, or animal cells under controlled environment. Such cells are used to produce medicines, enzymes, alcohol and biofuel. A bioreactor provides cells a safe, stable environment and makes available whatever they need to grow and work. The tank contains the sensors and other advanced control mechanisms that maintain the desire control environment in the tank. These factors include temperature, oxygen concentration, pH and mixing [4], [5].

1.1. Historical development

In the field of science and industry, bioreactors have long history. One use for bioreactors was during the war years. At that time, fermentation tanks produced antibiotics such as penicillin to fight World War II-induced infections where other methods had failed. [6] In 1970's era, bioreactors were used to produce insulin for people with diabetes from bacteria grown in tanks [7]. Thanks to these early-stage bioreactors, numerous lives was saved. Today bioreactors are used to grow cells from different systems. To produce human and animal vaccines as well as other drugs such as insulin, the bioreactor are being used on large scale.

1.2. Basic elements of a bioreactor

A typical bioreactor has following basic parts:

Vessel: It is the main storage tank, where biochemical reaction take place. In large tanks, it is usually made of corrosion resistant metal and can be clean easily.
Agitator: Agitators are the mixer used to material inside the tank uniformly.
Sparger: There is a device at the bottom of the tank where air or oxygen bubbles are let into liquid.
Sensors: These detect how warm it is, what the acidity level and oxygen content is inside the bioreactor.
Control System: According to the readings from sensors, controller automatically adjusts the circumstances inside the bioreactor.
Foam Control System: When foam is produced due to stirring, this system either gets rid of it or reduces it chemically or mechanically [8].

1.3. Types of bioreactors

Subsequently, a brief description of each type is given below depending on how it is used.

1.3.1. Mixing and aeration based

Stirred tank bioreactor: STR is a bioreactor which can be identified by characteristic of a stirred tank reactor where a rotating impeller is used to tank contents with end goal of bringing uniform dispersion of nutrients, cells and other structures in culture media. They are extremely scaled and varied meaning they can be used in biotechnology, fermentation process.

Airlift bioreactor: It has injected gases to circulate and mix culture broth rather than the mechanical shaking. It is also very suitable when culturing shear sensitive cells and with other straining tasks where vigorous mixing is not preferred.

Bubble column bioreactor: It rests on gas bubbles to stir and blend a part of a liquid medium which could be: a liquid that commonly carries cells or other microorganisms and it has no moving components. It is characterized by the fact that it is non-complex, high mass transfer and multi-purpose, to be used in fermentation and cell culture.

Packed bed bioreactor: A packed bed bioreactor is a bioreactor that has immobilized enzyme or microbial cells placed on a column or vessel typically a tube or cylinder. Such two-dimensional bioreactors can find a wide range of application in food processing, wastewater treatment and in tissue engineering as they enable high cell density and efficient mass transfer.

Fluidized Bed Bioreactor: It belongs to a subgroup of bioreactor in which that suspension of solid particles (which is generally an inert material like sand or activated carbon) are agitated by being moved either by the upward flow of a liquid or a gas. This makes a fluidized state where the particles behave as a fluid to facilitate good mixing and mass transfer inside the reactor. FBRs have received substantial industrial use in waste water treatment plant, and other industries due to the reason that they have potential biomass retention, high conversion rate and small size.

1.3.2. Biocatalyst type based

Suspensions cell bioreactor: This is another type of special designing of growing vessels that grow freely in some liquid media, unlike those adherent cultures in which some cells grow on surfaces. Such bioreactors play a critical role in mass cultivation of cells particularly where mass cultivation of cells is needed in the manufacture and treatment of biopharmaceuticals and research. They are characterized by such benefits as scalability that means they can be used to generate high volumes of cells or other cell products.

Immobilized cell bioreactors: These consist of immobilized cell bioreactors which are trapped in or on a support matrix and are capable of repeated use of catalytic activity and a positive aspect of free-cell systems. There are several ways in which such a kind of bioreactors is used which includes fermentation, making of enzyme and in biosensing.

1.3.3. On the base of reactor configuration

Tubular bioreactor: it is freely utilized in both liquid phase or gaseous form of reaction and could either be arranged vertically or horizontally. The advantages of the reactors of this kind are that it is easy to construct, easy to scale up and has high surface to volume ratio compared with other types of bioreactors.

Membrane Bioreactor: This is a type of wastewater treatment plant which consists of a biological treatment process which is accompanied with a membrane filtration process. Another good alternative that can conveniently replace the traditional clarifiers with the membranes in the separation of solid-liquid is an alternative that gives high quality effluent and a smaller system.

Photobioreactor: Photobioreactor is a reactor whereby photosynthetic organism is grown in artificial or solar light. These encapsulated photic reactors increase the environmental parameters of light, temperature and nutrient agitation which to permit the development of more biomass than in the natural systems. Photobioreactors are crucial in most of the applications which involve production of food, generation of biofuel and environmental cleaning processes.

1.3.4. On the basis of application

Anaerobic bioreactor: Anaerobic bioreactor is a device that degrades organic waste in the absence of oxygen, usually in a wastewater-treatment facility, using anaerobic microorganisms. It is among the technologies of the high-strength waste water treatment, biogas production form of renewable fuel and reduction of sludge mass.

Aerobic bioreactor: Aerobic bioreactor is a kind of biological reactor that oxidizes organic process load in waste water or any other waste material through the use of oxygen. It is a system which needed the use of aerobe microorganisms' ones that need the presence of oxygen to decompose the pollutants and any other contaminant present.

Hybrid Bioreactors: Hybrid bioreactors are the integration of different technologies and the biological treatment with a goal in mind of optimizing towards effectiveness and treat challenging types of a wastewater treatment or any other application. Most often they integrate the two, attached growth (biomass supported by some surface) and suspended growth (biomass in a free-flowing liquid). Some of the advantages that are brought about by this combination are that it increases biomass retention, the amount of pollutants that are eliminated as well as the ability to even be able to deal with variable circumstances with relative ease.

1.4. Application sizes of bioreactors

Small scale bioreactors shown in fig. 1 come with capacity of few litres. They are used in research and development and these are ideal for studying the effects of culture conditions on cultured cells physiology. For example, when you want to optimize parameters like temperature, pH and oxygen concentration.

Fig. 1 Lab scale bioreactor [54]

Pilot scale: A pilot scale bioreactor is a mid-sized vessel used for experimentation, in which process optimization and other trials can be done before scaling up to full size production volumes. Although the term "pilot" suggests only experiment, pilot scale actually refers to experiments that are conducted on a larger size than laboratory research but smaller in than manufacturing. It's used to validate the new process before staring the industrial production. A fig. 2 shows a typical pilot scale bioreactor that is used for trail purposes.

Fig. 2 Pilot scale bioreactor [55]

Industrial scale: The large bioreactors shown in fig. 3 can hold thousands of litres are actually factories in rough form. Their industrial use is to make things on a very large scale. Parameters like pH, mixing and oxygen concentration control become more difficult to achieve at the huge scale of industrial production.

Fig. 3 Industrial level bioreactors [54]

1.5. Common operational modes of bioreactor

The way in which a bioreactor is run can increase the efficiency of the process greatly. And depending on the operational mode that is selected businesses such as pharmaceuticals, food, energy and waste water is very important. Choosing the mode of operation there are several methods available for each different strength is accompanied by corresponding weakness. Some methods are very simple and good for small scale production. Others are more complex but allow continuous production of higher efficiency. Some methods give better control over cell growth and product formation. Others are more readily scalable on an industrial basis. The main types of operation depicted in fig. 4 are batch, fed-batch and continuous processes [9]. There are also some systems that are a bit of both or state of the art techniques such as perfusion or immobilized cell systems depending on many factors including product type, production volume, cost, time, and regulatory requirements.

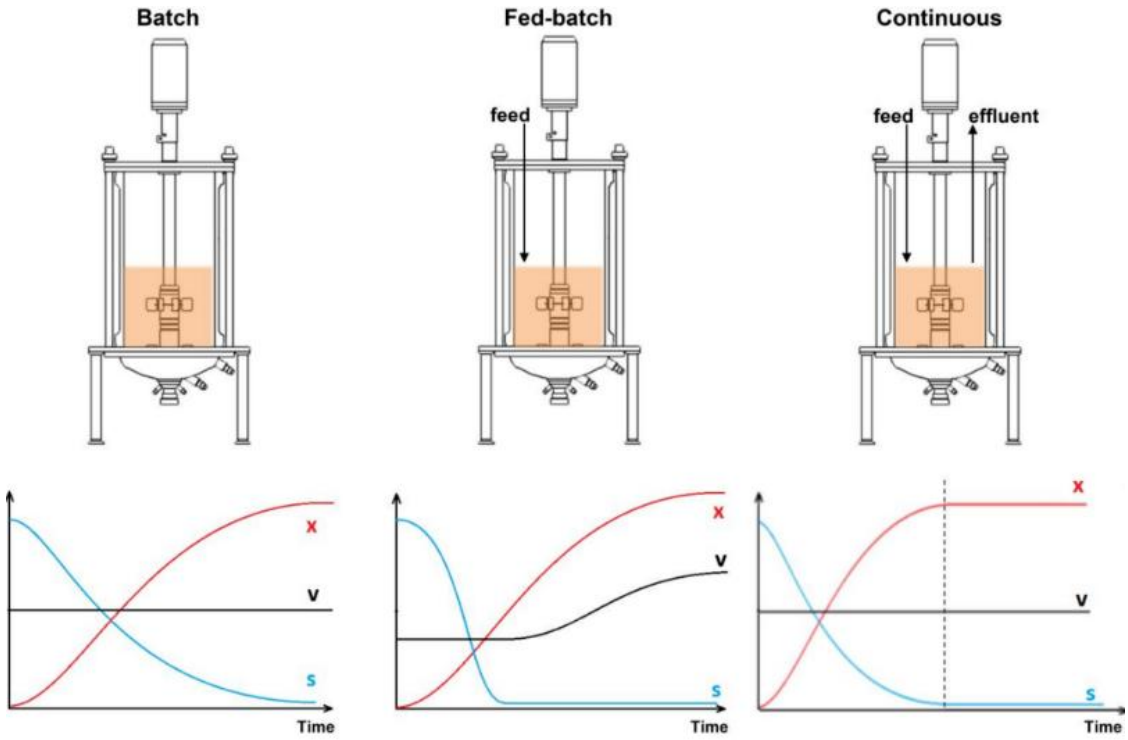

Fig. 4 Classification of bioreactors according to their modes of operation [11]

The most common operational modes are explained in following sections

1.5.1. Batch Bioprocess

As a mode of operating a bioreactor, a batch process is the oldest and simplest. When the reactor starts up, all the nutrients and microorganisms are put into it that would be required for the process to operate. Then, with the reactor closed, nutrients are no longer supplied, and the reaction is left to go on by itself. After a period of time, this process will be stopped and the product taken out. The reactor is next cleaned and readied for another batch. The system is easy to operate and control. It is mostly suitable for small scale to medium scale productions. Typical batch process has following steps:

1- Sterilize bioreactor and its equipment to rid it of any contamination.
2- Pour biochemical ingredients into reactor tank.
3- Process must be run for a fixed time.
4- Harvest from the reactor the product you want.
5- Clean and prepare for next batch.

Industrial Applications

Batch processing is very common in food and pharmaceuticals related process. Many antibiotics are made on a large scale in batch mode. For instance, penicillin is manufactured by the large-scale fermentation of species from Streptomyces in stainless-steel tanks. Products made by batch processing include other more specialized items, as in the following:

- Organic acids

- Amino acids
- Yeast and related products
- Specialty chemicals

Throughout the world, penicillin is an important antibiotic. The first step in the manufacturing process is to sterilize special nutrient solutions for the tanks. The mold is then added and allowed to stand five or seven days. During this time, penicillin is secreted out of it into solution. Then the liquid is filtered, and penicillin extracted and purified. The product quality is good using this method. On the other hand, a great deal of labour must be devoted to cleaning up each batch when using this mode of operation. Nonetheless, use of the batch mode is because quality is all-important in producing products and there needs to be strict control [10].

In batch process, there is no need for a separate control system, it is simply a matter of turning on the valve greatly simplifying maintenance procedures. Another advantage is their flexibility: with such a production method you can produce many different types of products at once they don't limit me to producing only single items at any given time. It is highly suitable for pharmaceutical production to ensure product quality, as the risk of external contamination is reduced because the entire process takes place in a closed chamber. They cost less than continuous processes to both build and operate. The fact of the matter is, however, that batch production also has limitations. They do not operate all the time, and therefore no production. After every batch run there is additional time for cleaning up and then setting it up again-one way or another, productivity is lost. The quality of each batch varies slightly: small changes in the process breed different results. in time, nutrients are used up and cells stop growing Further-more, each unit of product that is made is also accompanied by a larger amount of waste. The final reason is that it' s hard to make the system bigger, since if you want large tanks better mixing is needed and control becomes more complex [12].

Key Action Points

A few essential variables have to be monitored in order for a batch system to run smoothly. If our temperature is steady, there is no danger of the process failing. Just keep the pH value at an appropriate level and cells will thrive in such perfect harmony. In areas where larger tanks are employed this is extremely important: oxygen usually required by cells would be thoroughly depleted absent sufficient aeration or otherwise mixing poorly done. Bioreactor interior chemistry is such that any air pressure causes frothing, and so anti-foaming agents are used to quell problems. A number of tools, including sensors, are employed to measure levels of things such as temperature, pH and the amount oxygen in the atmosphere. Only in this way can workers see that everything is running as it should [13], [14].

1.5.2. Fed-Batch Bioprocess

Fed-batch bioprocessing is one of the most popular operating models used in industrial biotechnology. It unites aspects of continuous and batch production. In fed-batch, the process starts off as a batch but when nutrients are low, fresh nutrients are added after a certain season instead of being cast aside and the cells with it. This helps to prolong the production period and so increases the yield of products. Fed-batch processing is advantageous for breaking cop-off poisonous products that would, under other circumstances, destroy growth of biomass and nutrition concentration. It also

meets the presently growing number of high-value added bioplastics like therapeutic proteins, enzymes and biofuel. Fed-batch bioreactor: This kind of bioreactors starts with a small sum of feedstock like glucose or nitrogen source. And additional feed is gradually added during the process. The feeding mode is decided by the microorganisms, the objective of the process and the product. Examples of used feeding operational modes comprise:

- Constant rate feeding
- Exponential feeding
- Feedback-based feeding

When process is finished, the product is taken out from the reactor.

Industrial Applications

In industry, fed-batch systems are used to produce a variety of important antibodies e.g. mAbs are made in fed-batch reactors with mammalian cells such as CHO (Chinese Hamster Ovary) cells [15]. In the same kind of reactor, recombinant insulin and growth hormone can be created as well as human interferons [16]. Xylanases (enzymes that break down xylan), lipases and cellulases are also prepared in a fed-batch process. Fed-batch processes are ideal for producing such amino acids as L-lysine and L-threonine, and can turn sugars into alcohol over an extended timeframe via the yeast Saccharomyces cerevisiae. In vaccine development, fed-batch process is popular among vaccine manufacturers since it permits both strict control over cell growth and production.

Monoclonal antibodies find application in cancer treatment, diagnosis and auto-immune diseases. These kinds of antibodies are usually produced with animal cells such as CHO. In typical a mAbs fed-batch process:

- pH and oxygen are monitored using controller.
- Cell growth and biomass concentration is monitored using online sensors.

This approach results in high-density culture and concentrations greater than 5 g/L of mAb in today 's bioreactors [15]. A fed-batch bioprocess has many advantages. It lets the cells grow longer, achieving higher product yields as a result. The way nutrients are used is controlled, so there are no problems with overflow metabolism that can happen when you give too much sugar at once. By feeding nutrients slowly, it cuts back on unwanted by-products such as acetic acid which forms in E. Coli cultures. However, in fed batch, the control of feeding conditions and rates is very delicate, so this process can get complicated. This method relies heavily on sensors for pH, oxygen and nutrients control and those sensors can fail or give false readings. Feeding causes the formation of foam as well, which needs to be managed with anti-foaming agents. Finally, as the product is all collected at once it may be more diluted than in continuous systems [17].

To successfully run a fed-batch bioprocess, a seamless plan is required. In particular, all the feeding stuff, nutrients in particular, needs to be sterile to prevent contamination. By gathering data from past experiments and also using mathematical models, the feeding schedule is carefully worked out [18]. Foam control is necessary to prevent any kind of error, without some kind of control system on the

tank, this can become a mess pretty quickly. Real time monitoring with software and control systems helps adjust feeding to the process

1.5.3. Continuous Bioprocess

Bioprocessing with continuous mode is based on instant media percolation into the fermenter, at the same time culture is pulled out of reaction broth, containing cells and products transported off with it. This keeps a constant working volume while keeping cells in an active growth state. The continuous life-support systems are designed to make the entire process maintain an existent steady state, with a stable balance over time between cell growth, consumption of nutrients by cells and product formation. Compared with batch and fed-batch systems, continuous operation can increase the overall production time be more conducive to cooking quality control, it also enhances productive output per unit resource consumed and is particularly well-paid by biopharmaceuticals and industrial biotechnology practitioners. In general, continuous bioprocessing reporting takes the form of a chemostat, with dilution rate (i.e. fresh medium addition per unit bioreactor volume) and rate of flow kept constant. Other types include turbidostats, which use optical sensors to keep the biomass concentration constant, in perfusion systems cells are retained with filters or membranes while they jettison spent media and product.

Industrial Applications:

More and more industries are using continuous bioprocessing. In biologics production for instance, it provides an assisted means of producing monoclonal antibodies and vaccines. By the techniques of the perfusion system, as that applies to wastewater treatment, it is now on a continuous basis. Also, the cost of raw materials for manufacturing bio-ethanol falls when using the yeast bioreactor, with a reduction in waste as well. Meanwhile, with the assistance of process analytical technologies as well as automation, a great many pharmaceutical manufacturers are taking their entire process for the continuous manufacture of drugs from now on.

From the industrial point of view in continuous bioprocessing, cells are active in their growth phase longer than they would normally be, which increases production time for a working product while saving on Titre. At the same time this general trend also leads to more productivity increases, you get more kilograms of product over any given time because the operation goes on without stopping. In case of disorientation, this approach maintains a strict and even consistency as the production process continues. So instead of stopping frequently to decontaminate or change installations, these systems not only save time but also remain trouble free. They can also be more efficient: thus, a small bioreactor with equivalent results to a larger one in batch processing conditions continuous bioprocess not only fits into the laboratory, but also goes well with downstream processes [19].

The longer any system runs, the more opportunities there are for contamination to get in. This may infect a whole batch. Microorganisms used in the process might change genetically over time which can lower productivity or change the product. Systems like these really require highly advanced technology and automation to keep everything going smoothly However, in the pharmaceutical industry regulatory environment may make it difficult to get approval for continuous operations.

Critical operational factors:

For continuous bio processes to be successful the many disparate elements must come together and work seamlessly together to deliver stable long-term high efficiency operations. Methods such as spin filters, hollow fibre membranes and acoustic settler allow cells to remain in the bio reactor and select all undesirable materials.

The bioreactor is designed to support stable growth of cell. Continuous monitoring of important parameters like glucose, lactate, ammonia etc. is important to maintain optimal conditions inside the reactor. In most of those places, automation play an important role to keep the environment under controlled condition. They also feature strong cleaning systems such as CIP (clean in place) for long runs to keep the cleanliness of environment. These elements are very important in these biological processes to maintain the stability of process [20].
A comparison of the three processes batch, fed-batch, and continuous is summarized in Table 1, while their graphical representation is illustrated in Figure 5.

Table 1. Comparison of batch, fed-batch, and continuous bioprocess

Criteria	Batch	Fed-Batch	Continuous
Productivity	Low to moderate	Moderate to high	High
Product Quality Consistency	Variable between batches	Improved over batch	High and consistent
Operation Time	Short (cyclic)	Medium (semi-continuous	Long (steady-state)
Risk of Contamination	Low	Moderate	High (due to long runs)
Control Complexity	Simple	Moderate	High (requires automation)
Capital Investment	Low	Moderate	High
Process Flexibility	High	Moderate	Low to moderate

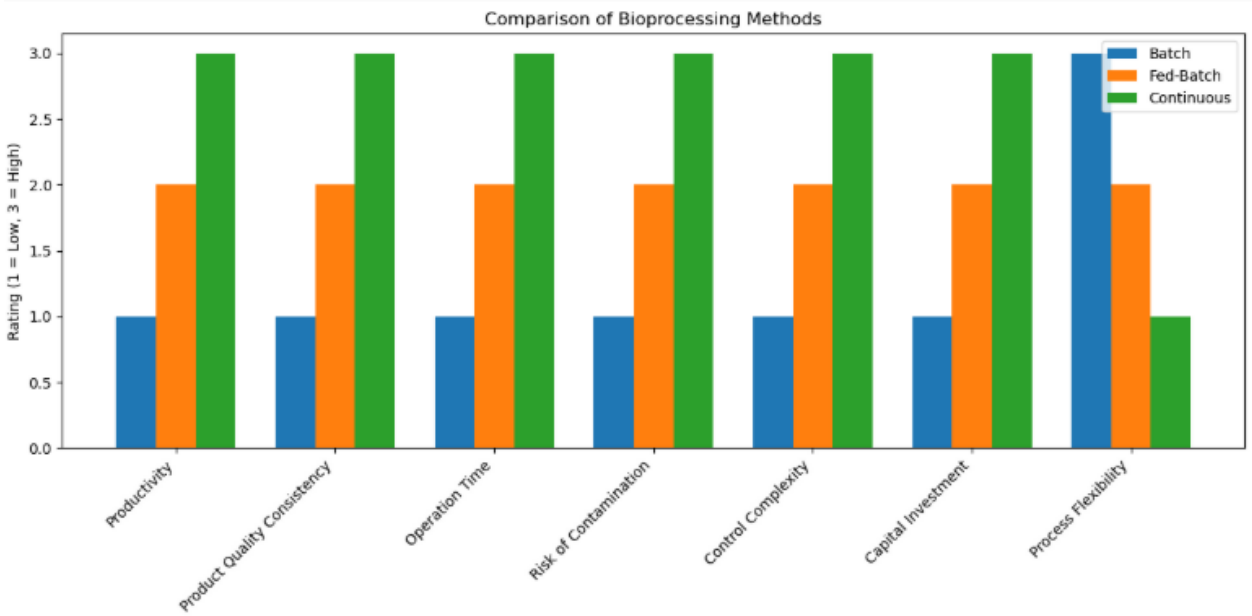

Fig. 5 Comparison of batch, fed-batch, and continuous bioprocess

1.6. Major Industrial Applications of Bioprocess

1.6.1. Fermentation Processes

Fermentation involves the biological process of conversion of useful products through the sugar or other nutrients using the microorganisms. This is done under a controlled environment in a bioreactor; this is in an environment that has the correct temperature, oxygen, and pH. Production of antibiotics like penicillin, streptomycin and erythromycin is highly referred to through bioreactors. These are antibiotics which destroy harmful bacteria inside body. Indicatively, penicillin is produced by the use of Penicillium chrysogenum. This fungus is cultivated in large bioreactors together with sugar and other nutrients to obtain the drug. The environment within the bio reactor is maintained to produce a high percent of antibiotics. Alcohol such as ethanol is fermented with yeast sugars. It is done in bioreactors in the food and fuel sectors. Ethanol is applied in food as a drink (in beer and wine). It is employed in production of bioethanol that is blended with gasoline in fuel production [21]. Enzymes are those proteins that accelerate reactions. They find application in most industries including food, medicine etc. In producing bacteria or fungi used in making enzymes such as amylase, protease and lipase, bioreactors are employed. Those are extracted and purified to be used [22].

1.6.2. Cell Culture Applications

Cell culture refers to maintaining animal or human cells in some specified conditions and bioreactor is used as a system to multiply the cells to manufacture medicines and vaccines. The process of vaccines manufacture consists of viruses or bacteria that are grown inside living cells, preventing diseases. Such cells can be Vero cells, or CHO (Chinese Hamster Ovary) cells, and can be grown in bioreactors to make vaccines against diseases such as polio and influenza, or SARS-CoV-2 (COVID-19) [23]. Monoclonal antibodies are unique proteins which are applied in curing ailments such as cancer and autoimmune diseases. The mammalian cells are used to produce this type of antibodies e.g. CHO cells. Bioreactors assist these cells to multiply in high quantities as well as in stable conditions hence large medications of the medicine can be manufactured [24].

1.6.3. Biotransformation Processes

Biotransformation refers to the modification of an entity through the employment of cells or enzymes into a different more usable agent. There are microorganisms and enzymes that are able to convert low cost raw materials to products that are valued. As an example, the steroids may be converted into active ones with the help of the specific fungi. This is carried out in bioreactors in an environment that helps the activities of the enzymes within the cells. Natural flavours and fragrances also are prepared by biotransformation in bioreactors. It involves the synthesis of vanillin with the input of ferulic acid and raspberry aroma with the input of glucose by use of yeast and bacteria [25]. The artificial products are not as accepted as the natural products in the food industry, as well as in the industry of cosmetics.

1.6.4. Wastewater Treatment

In the waste treatment of water, bioreactor reacts with water by neutralization of dangerous substances with the help of microorganisms. In activated sludge process, the breakdown of organic pollutants in the wastewater is done through bacteria. The bioreactor is used to introduce oxygen and mixing to

nurture the bacteria to grow and work effectively. The kind of method is mostly applicable in municipal and industrial wastewater treatment plants. In anaerobic digestion bacteria degrade waste in the absence of air and forms biogas, primarily, methane. As a source of energy, this biogas can be utilized. The treatment process is used in the treatment of the sewage sludge and food waste in special bioreactors known as the anaerobic digesters. Membrane bioreactors integrate biological treatment and a membrane filter, so that pure water can flow through the filter whereas, bacteria and solids can be held by it. The MBRs are deployed in the case of cheap water needs to be available following the treatment [26], [27].

1.6.5. Biofuel Production

Biofuels are also products of bioreactor; these are fuels that comprise of living organisms such as plants or other microorganisms. They are bioethanol, biodiesel, and biogas. It has been said above that bioethanol is produced in a bioreactor where yeast ferments sugar contents (corn, sugarcane, or lingo-cellulose biomass). It is used as a substitute to gasoline in vehicles [21]. Biodiesel can be produced by conversion of plant oils or animal fats in presence of microbe or enzyme to enable it to undergo chemical reactions labelled transesterification. The enzymes or the process are produced in bioreactors so that the process is performed under clean and controlled conditions [28]. Originating in anaerobic bioreactors as a mixture of methane and carbon dioxide, biogas is created out of organic trash. It is applied in cooking, electricity and heating both in the rural and urban surroundings.

1.6.6. Other Applications

There are also other numerous applications of bioreactors. Microbes are applied in the food industry to make yogurts, cheese, vinegar, and soy sauce in the bioreactor. Fermentation is done especially in a tightly controlled way to ensure quality and taste [29]. Bioreactors are used in environmental work: soil bioremediation takes place when oil spills are cleaned up by microbes, and soil is freed of poisonous chemicals. It also is applied in air biofilter to destroy toxic gases such as hydrogen sulphide and volatile organic compounds [30]. Bioreactors are useful in agriculture in the manufacture of biofertilizers and biopesticides. The products promote the growth of plants and thus minimize the use of chemical fertilizers and pesticides.

1.7. Modelling techniques

The modeling of biochemical and biotechnological processes in the bioreactors is an important tool in the investigation, prediction, and the optimization of the process. Such systems are also, in most instances, related to existence of living micro-organisms, elaborate biochemical processes as well as non-linear coupling of the physical, chemical and biological components. Since the contemporary management and monitoring of all the inner circumstances of a bioreactor are typically not achievable, models are incorporated to define bioreactor mathematically and therefore that the operators and controllers ascertain the most avoiding method of operating it. To meet the current industrial requirements concerning the quality of their products as well as regulation laws of their products on new highs of accuracy and reliability of models, there is no doubt that it is more than ever needed [31].

Some of the areas that bioprocess modeling is utilized are in pharma industries, bio-fuels, food industry, wastewater and enzyme production. The models find assistance in estimating of various important process parameters such as biomass concentration, rate of substrate consumption and product yield among others which would have been very expensive or time consuming to be measured directly. In the current bioprocess engineering, modeling is also used as a means of control, fault detection, optimization and process scale-up in addition to the simulation. This is what places modeling as the bridge between exploratory scaffolding and intelligent decision making in full scale production levels environments [32].

Generally, we may distinguish between three favourite modeling techniques that are applied to such diverse an entity as biochemical and biotechnological systems, i.e., mechanistic (kinetic) models, data-based ones and hybrid models. Each of these methods has its merits and demerits depending on the kind of a process, amount of the data and requirements of the modeling ends.

And regardless of how you do it, some of the main steps in construction of the model occur. They might be specification of system boundary, variables of interest, available mathematical correlations, formulation of parameters, verification of the model considering actual experiment or real-life data.

1.7.1. Kinetic modelling

Kinetic models are valuable instruments and it narrates biochemical processes and biotechnological process movement behaviour during the activity in bioreactors. It is kinetics of reaction, and by mathematical models, it describes the uptake of organisms, usually bacteria or fungi, the use of the substrates and a product is generated over a period. Under this kind of modeling it is possible to give a description of the biomass growth, substrate consumption and the end products together with by-products in different reactor conditions.

This can simply be explained by Monod kinetics in which the rate of growth depends on the concentration of the substrate. In this model, both substrate limitation and excess are given all importance in inhibiting the growth. Monod equation has been further generalized to encounter the idea of substrate inhibition, product inhibition, and the possibility of numerous substrate inhibitions. A set of parameters which characterize these models are namely; maximum specific growth rate, saturation constants and yield coefficients, and are obtained very specifically through laboratory experiments [32].

The benefit of the biology and chemistry basis in kinetic models is also present. These translations provide a researcher and engineer more understanding of a system, laboratory data figure scales, and reduce the process design time. As an example, the same process of scaling up a fermentation process involving transfer of a fermentation process between a small lab flask to an industrial fermenter relies on kinetic models to generate an understanding of how the systems will behave under various conditions e.g. pH, temperature and agitation speed.

However, the drawbacks associated with the kinetic models are that they are not minimum. The biggest problematic factor is that there are not much credible experimental dates of the parameter estimation of the model. Biochemistry is sloppy work and it might work a bit different with different organism or it might even be a little bit different on the condition itself. There is also the change of

systems over time and so this would amount to a poor prediction of long-term behaviour using a stationary model. Secondly, the model would ignore the effect of uncertainty when the processes are conducted such as incomplete mixing and equal distribution of temperature due to large storage time in industry, which would reduce the accuracy of prediction [33].

Many processes in order to be called bioprocesses, must only consist of chemical reaction but also must entail mass transfer, heat transfer and gasses exchange. As an example, within aerobic fermentation, the amount of oxygen consumed by the microorganisms so far needs to be added to the total diffusion of the gas phase. Without taking into consideration the limits of oxygen transfer, a model, based solely on the reaction-rate, will be completely misleading. By this way, the kinetic models which include the physical transport model and chemical kinetics model are more advanced to be implemented in reality, particularly the reactor with industrial scale.

In bioprocess engineering there exist two families of kinetic models, the unstructured and the structured models. Unstructured lumps the above-said biomass of the cell into a single variable and do not take any internal organelles and parts in account, enzymes, etc. These models of structures are simpler, they are manufactured in directly of most industrial processes since they were computationally easier to perform and comprise the fewest parameters. Instead, the structured models contain internal elements that refer to the cell and have more intuitive understanding of the relations between metabolism and gene expression. They are more complex and find use in the applications of the areas of engineered organisms and the metabolic engineering.

Kinetic models of different reactor systems, batch, fed-batch and continuous systems, have been studied. In the case of batch reactors, we can know how long the reaction will take and the optimal time to shut the process using the model. That is why in a fed batch process (actually this kind of system is very popular in industrial fermentation) the usage of kinetic models was suggested to generate the feeding policies with the final aim to avoid the substrate inhibition and to maximize the yield. Kinetic models then are the basis of the determination of the optimum flow rates of the continuous reactor so as to prevent biomass wash down [34].

Kinetic Model Examples

For example, Song et al. [34] proposed kinetic equations for fed-batch fermentation processes based on a modified Monod-type models. A substrate inhibited Monod model was used to describe the microbial growth rate:

$$\mu = \mu_{max} \frac{S}{K_S + S + \left(\frac{S^2}{K_I}\right)} \qquad (1)$$

Furthermore, product inhibition effects were incorporated as:

$$\mu = \mu_{max} \frac{S}{K_S + S + \left(\frac{S^2}{K_I}\right)} \left(1 - \frac{P}{P_d}\right)^i \qquad (2)$$

In addition, product formation kinetics were modelled using the Luedeking–Piret equation:

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X \quad (3)$$

Where

X: Biomass concentration (g/L)
S: *Substrate concentration (g/L)*
P: Product concentration (g/L)
μ: Specific growth rate (1/h)
μ_{max}: Maximum specific growth rate (1/h)
K_S: Half-saturation constant (g/L)
K_I: Substrate inhibition constant (g/L)
P_d: Product inhibition constant (g/L)
i: Empirical exponent representing product inhibition effect
α: Growth-associated product formation coefficient
β: Non-growth-associated product formation coefficient
t: Time (h)

1.7.2. Data driven modelling

Data driven models handle nonlinear dynamics as well as time varying and complex nonlinear dynamics in a better manner in the world of bioreactors. Such models can be used in order to obtain the relationships between the inputs e.g., feed rate, temperature, pH, dissolved oxygen and the outputs e.g., biomass, product concentration, enzyme activity through statistical as well as machine learning methods.

Feedforward neural networks (FNN) recurrent neural networks (RNN), support vector machine (SVMs), random forests (RF), and gaussian processes (GPs) are some of the common data driven techniques. Neural networks are usually applied in practice since they have the ability to model nonlinear relationships and linear mappings and at the same time the temporal relationships. LSTM (long short-term memory) and similar recurrent networks can learn the temporal dynamics in time series of data, which in turn may be used to predict subsequent states, e.g. in substrate utilization or product formation. Other ensemble approaches such as random forest achieve good results as well and also capable of ranking the variables and this could be important as far as the monitoring of the process and feature-selection is concerned. The reason behind the choice is based on the quantity of the data, the computational resources and real-time limitations.

Among the outstanding benefits associated with the data driven modeling is its fluidity and flexibility. Using new data models can be re-trained or fine-tuned to answer genetic or environmental perturbations to the bioprocess. This flexibility is of special importance in terms of early notifications, quality of oversight and model improvement. However, the reality is this; you can go so far only with the data-driven models. They demand large quality data sets that are needed to assemble the entire process operating space which is usually not common to early or novel processes. Another risk is overfitting in which the model has learned noise as well as pattern with small data sizes. Interpretability is problematic most of the time.

Nevertheless, the other issue is the scale up. Laboratory scale derived models used in modeling might not work in industrial scale reactors since the mixing, oxygen transfer/temperature profiles are changed. Such a relative dependence of the scale brings down the accuracy of pure data driven approaches. Some of the ways to mitigate this effect are: transfer learning where by models are trained on data at one scale and the adjusted to smaller scales or inclusion of penalty terms to prevent exclusive use of scale dependent features when training models [35]. However, data driven models have been deployed in batch or continuous operation in a measured way. In the fed-batch production of antibodies using Chinese Hamster Ovary (CHO) cells; neural networks have been applied to predict viable cell density and product titre (using real-time measurements given by sensors). This aspect allowed a complex or staged adjustment of feeding rates to maximize productivity and maintain life of animals [36]. Moreover, ensemble learning was able to model anaerobic fermentation of biogas composition with the varying substrates successfully, and has the potential to give an intelligent feed control system in order to optimize energy production.

1.7.3. Hybrid modeling

In a recent past, a hybrid modeling has been getting a score in the study of biochemical and biotechnological systems. These models are the combination of two kinds of approaches; one is the data-driven methods which are represented in terms of data like neural networks, and another one is the models relying on the scientific laws like mass balances. This is how hybrid models will get the best of both worlds. They absorb the properties that are understood of the physical systems through equations and which can be trained on physical data to learn properties that are unknowable, or even shifting, of the underlying components.

In the case of the industrial bioprocess, it often happens to be that, not the whole process is known very well. An example is of clear chemical reactions or biological processes which constitute a system but there is much that we do not readily express in the form of equations. These and others raise the issue of variability in equipment's, the noise measurement, the changing environment which is fluctuating, since it is a proven fact that cell tendencies alter with time. These unknowns can be handled far better with a hybrid model than with a simple model define by equations only or by data only. Among such methods that have recently been popularized is the so-called physics-informed neural networks (PINNs). They are neural networks which when trained contain the actual physical equations. This enables the model to approximate the actual behaviour of the real-world even in the presence of noise as well as missing data. In the case of fed-batch culture of CHO (Chinese hamster ovary) cells, hybrid PINN-based the modeling of fed-batch CHO cells cultivation is introduced in one article [11-37]. The based cell growth and substrate were described by a mechanistic component and the rest part was predicted by neural network based on industrial data. The consequence of this is that the combination of a data-driven prediction with a model prediction out works than both methods of prediction using the equations as well as using the data-driven models [37].

Hybrid models provide a lot even in the terms of scale-up of bioprocesses as well. Factually, processes are normally brought into scale to be tested in tiny reactor laboratories. However, the same is not necessarily true on a scale that is more industrial within the reactors. This is the case as a result of a difference in environment, blending, heat, and concentration of oxygen. Model trained on pure data alone in one scale cannot work in a different scale. Another hybrid model was developed using the shake flask data and was tested on 15L reactor. It can correctly observe the number of viable cells

and the product with a percent error of less than 22 percent that is an acceptable value. This demonstrated how the hybrid models had an ability to traverse at scales with more precision than other modes of models [38].

The hybrid model has various advantages. To start with, the focus of the model is equations derived out of physical laws and because of this fact, the model fits so well to the domain of physics that the model requires less data to be fit against, say, the model based solely on data. It is convenient on bioprocess data when it becomes expensive and slow. Second, it contains physical data, which allow the model to provide sensible answers, regardless of the operating conditions. More suited than past oriented models of coping with the new. The third reason is that the findings of the mechanistic model are simple to report and interpret by the regulators or operators of the plant since its parameters are with physical meaning. Such openness in the model usually makes the hybrids more acceptable in the regions where quality has a stringent criterion. Even hybrids building of model may be more challenging. The first one is the way to unite the mechanistic and the data-driven aspects. Robotic applications in other applications, the equation-based model output feeds the neural network squad. In others, the two processes are in parallel and their output integration follows. The second one may be too complicated or unstable in case the first one is too difficult. Another complication besetting hybrid models, however, is the fact that they have to adjust internal parameters as prudently. This contains such details as a size of the layers of the neuro network, training rate, training data. When mis-selected, these may render the model to be in-effective.

2. Role of pH in industrial bioprocesses

pH shows to which category acidic, neutral or basic a solution belongs. It is a question of life and death in bioreactors. Most of the bioprocesses involve the use of living cells or micro-organisms to manufacture something. A living organism is extremely sensitive to pH. When the pH is not correct the cells will grow or will not produce the desire product. In this part the reasons of why we should control pH in the bioreactor as well as what will happen when we have a poor control of the pH will be described.

2.1. pH influence on growth of microbes

Bacteria, fungi, and yeast all these are microorganisms which prefer to grow at some particular pH range. Each microorganism possesses the optimal pH, within which it grows fastest. Growth is slow or halted by a low or too high pH. To take an example, yeast cultures which are used in the production of ethanol grow best at a pH of 4.5 -5.5. The growth rate will reduce in the pH levels of below 4 or higher than 6 and the Ethanol yield will be reduced [39].

2.2. Influence of pH in the Enzyme Activity

Enzymes hasten the rate at which chemical reactions take place and they are proteins. Activity of enzyme in the cells of cell is essential to a variety of bioprocesses. Enzymes are also optimum at certain pH levels. The enzyme will die or denature in case the pH is too high or too low.

As another illustration, the enzyme amplitude, which is used to divide starch, works optimally at pH 6. Enzyme activity is less or has been denatured at pH of 4 and 8 [40]. And this is equivalent to producing less of the product. The presence of a suitable pH can help to maintain the enzyme in an active as well as efficiency state in the bioreactor.

2.3. Effect of pH on Product

In most of the biotechnological processes the pH-sensitive product is resistant to any pH assumption. Ineffective control of pH might find an individual in a position of product degradation, isomerization or no formation of a product. When making vaccines in mammalian cells the pH is kept between 7.0 and to 7.4. In case its tips, stress proteins may be lost out of cells, they may cease to grow, or die. This has a consequence on quantity and quality of vaccines [41]. The other example is in the lactic acid fermentation. Therefore, the bacteria like Lactobacillus produces the lactic acid. Should pH become too low (which is reached should it not be regulated), the bacteria would no longer produce acid and thus may die. Hence such alkaline solutions like sodium hydroxide (NaOH) are added in order to keep the constant pH level through the reaction [42]. The optimum pH ranges for different biotechnological processes are given in table 2

Table 2. The optimum pH ranges for different biotechnological processes [43]

Biotechnological Process	**Optimal pH Range**
Ethanol fermentation (*yeast*)	pH 4.5–5.5 [27]
Penicillin production (*Penicillium chrysogenum*)	pH 5.0-8.0 [31]
Enzyme production (*amylase, protease*)	pH 6–8 [28]
Vaccine production (*mammalian cells*)	pH ~7.2 [29]
Lactic acid production	pH >5.0 [30]

In all these processes, tight pH control is necessary for good results.

2.4. Advantage of fast pH regulation

When pH is good, the following happens:

- Product yield: create more product using the right pH.
- Quality of products: the quality of the products is pure and stable.
- Process: The system never falls to the bottom.
- Cell health: Cells develop better and lives longer.

A high number of modern bioreactors use pH Automatic control. The system would involve the use of sensors, pumps, controllers among other relevant modern techniques that will help to maintain pH to the predetermined level.

2.5. pH control techniques

The control of pH in bioreactors is a mandatory condition of effective operation of biochemical and biotechnological processes. pH is of great significance to microbial responses, enzyme activity, product yields and metabolic routes. A slight change in pH may change the rate of cell and product formation in a bioreactor. Thus, keeping the pH at an intended setpoint is a key requirement of industrial processes (such as fermentation, bioethanol manufacturing, enzyme production and wastewater treatment). Over the years there are several measures of control that have helped enhance control of pH, and each with their advantages and disadvantages. Widely employed methods offer PID regulation, adaptive regulation, fuzzy logic management, model predicative control (MPC) and neural system, statistical methods and model-free adaptive controls (MFAC).

2.5.1. PID control

PID control is a control strategy which uses a feedback control mechanism, and uses a continuous calculation of errors as the difference between the desired setpoint (defined pH) and a measured process variable (the current pH). It uses a correction, which comprises of three terms:

Proportional (P): It will compensate the proportion in accordance to its magnitude.
Integral (I): It functions by eliminating a residual steady-state error by means of integrating the past errors.
Derivative (D): It forecasts or predicts error by the rate-of-change.

The technique is very common in bioreactor since it is simple and shows real time response. Nevertheless, the physical process of pH control shows non-linearities, and this aspect in particular becomes problematic to traditional PID controllers [44]. The fig. 6 shows the structure of pH neutralization process using classical PID control.

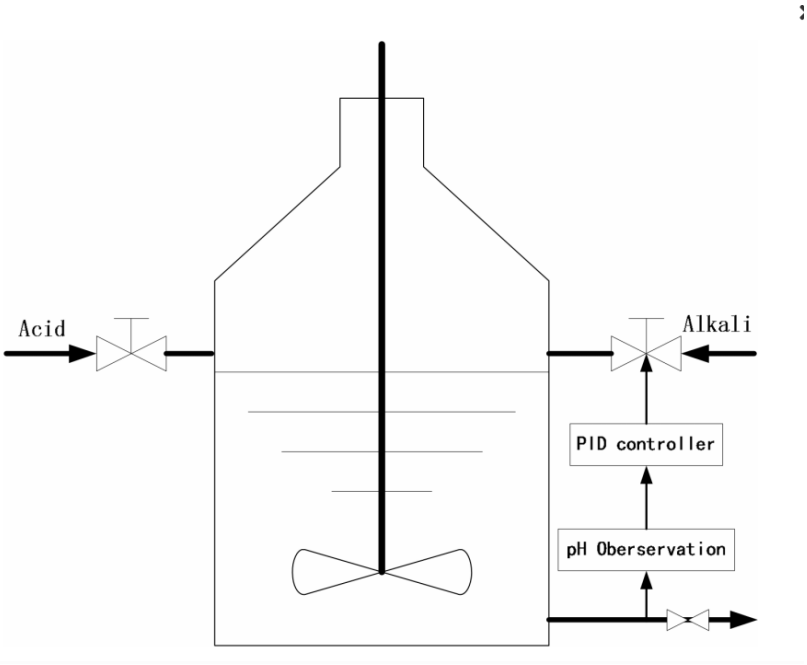

Fig. 6 The structure of pH neutralization process using PID control [44]

The simplicity of the operation, ease of application and common acceptance in the industry makes PID control to have a number of advantages in bioreactor pH regulation. It does not need an elaborate mathematical description of the bioprocess that is valuable in biological systems in which dynamics are complex and not well understood. The proportional part immediately corrects the system depending on the error at the present moment, the integral part cancels steady-state error by considering the sum of the error which has already occurred, and the derivative part predicts the undesired error anticipating the trend of the future, increasing system stability. Such characteristics give PID controllers by far the highest responsiveness and ability to control pH to very tight tolerances, which is essential in order to ensure optimum microbial or enzyme activity. Besides, PID controllers are computationally cheap and that they can be implemented in most industrial control systems, making them cost-effective to resolve real time control on large scale bioreactors [44].

However, there are also many drawbacks to PID control, such as the pH dynamics, which is nonlinear and time varying in bioreactor environments. The pH-neutralization process is nonlinear, particularly in the pH-neutral (pH neutral = 7) region: adding just a small amount of acid or base can cause a significant change in pH. The conventional PID controllers that are linear may not be able to preserve stability and precision in such zones, thus resulting in overshoot or oscillations [44]. Moreover, PID controllers are tuning sensitive; when the gain is not appropriate, then the performance may be compromised or worse still the system may be caused by instability. The process of tuning manually is time-consuming and might not cope with the changing process conditions e.g., changes in microbial

metabolism or feed composition. Inability to work in complex bioprocess environments has led to the formulation of more improved forms of PID and hybrid controller algorithms, including: neural network based assisted PID controllers, that strive to be more adaptable and robust [44].

2.5.2. Adaptive control

Adaptive control shown in fig. 7 is the control strategy whose parameters are calculated in real-time dynamically to adapt to changes in the dynamics of a system, or to new environmental circumstances. It works using the knowledge that the structure of the system is understood, and some of those parameters are unknowable or change with time. Adaptive control makes changes to achieve desirable measures by constantly checking the system performance to achieve these measures. Popular ones are Model Reference Adaptive Control (MRAC), a strategy in which a reference model is matched with the output of a system using error minimization; self-tuning regulators (STR), a strategy that finds and updates the parameters of a system on-line; and gain scheduling a strategy in which a system switches between defined sets of controller gains in response to operating conditions.

Adaptive control has a number of attractions to bioprocess use. It is adaptable to time-varying and nonlinear dynamics than controlled fixed-gain systems such as PID. This renders it appropriate to those processes in which the system dynamics transform with time as a result of microbial growth, consumption of substrates, or adapting to changes in metabolism. Even such fluctuating conditions can be tightly controlled by adaptive controllers resulting in increased product yield and better process stability [44]. But these advantages are associated with more complexities. Adaptive systems need to capture real time measurements properly and have good estimation algorithms which may become computationally demanding. Besides, an incorrect adaptation or inappropriateness of the models may result in instability or worse performance. In others, the adaptation mechanism can be either too slow or too reactive leading to oscillation or the overshoot. Nonetheless, the problem of advanced bioprocess control using adaptive control may be addressed through the intervention of a combination of adaptive control with techniques of nonlinear models or hybrid methods [46].

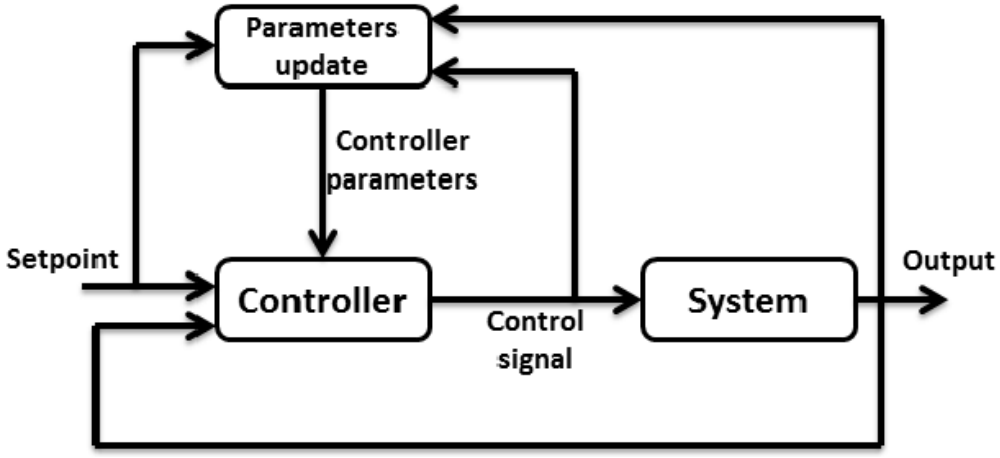

Fig. 7 Block diagram of Adaptive Control system [45]

2.5.3. Fuzzy logic control

Fuzzy logic control (FLC) Linguistic variables and Fuzzy sets have been used to replace the exact numerical quantities in a rule-based control strategy in fuzzy logic control that achieves verbal

reasoning and thinking in human beings. Fuzzy logic can be applied in such domains as regulation of bioreactor pH, where the nonlinear and imprecise characteristics of biological systems can be accommodated without necessarily defining a precise mathematical model. An average fuzzy controller comprises of fuzzification interface, a rule base and an inference engine and a defuzzification interface. To take an example [47] used a Mamdani-type type fuzzy system to model the dynamics of a strong acid strong base neutralization process. The fuzzy system also used the knowledge of experts together with real-time measurements; hence the controller was able to maintain flexibility to the changes in process conditions. Fig. 8 present the schematic diagram of pH control process using computer control system.

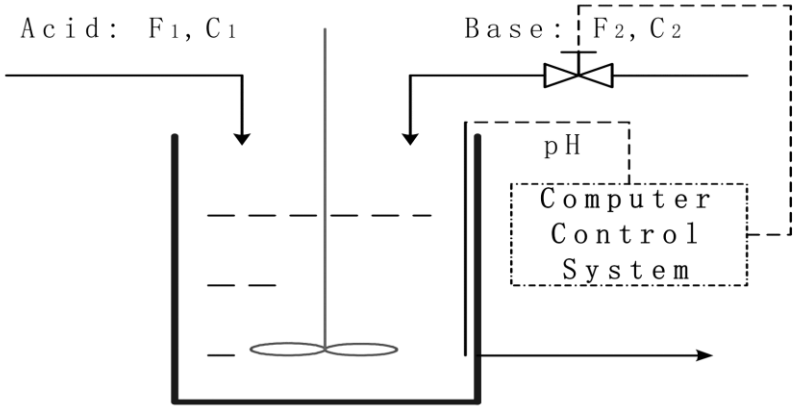

Fig. 8 A schematic diagram of pH control process [47]

Fuzzy logic control has a couple of advantages in bioprocess applications. It is naturally resistant to the uncertainties and nonlinearities of the system and therefore suitable to the control of pH, which presents a strongly nonlinear titration curve, and which is easily perturbed. Unlike process-based controller, PID or model based, FLC does not need a good process model to operate, making it easy to implement and less reliant on system identification. Further, fuzzy controllers are able to incorporate expert knowledge by imposing pool of linguistic rules which allow direct design and tuning [47]. Fuzzy logic control is however limited. The consequence of the quality and completeness of the rule base in the performance of the controller is very crucial and therefore in most cases the construction of the rule base is heuristic in nature. The process of creating a good rule set and membership functions may consume time and it is subjective. Also, fuzzy controllers are adaptive but when working in very dynamic conditions, and without the inclusion of adaptive mechanisms or learning algorithms, they may not work best. In other instances, the absence of formal stability assurances can be an issue as well in bioprocesses that are safety related [47]

2.5.4. Model predictive control

The model predictive control (MPC) is an effective control that is based on dynamic model of the process to predict the future system behaviour and summarize the actions to control the process in an optimal way in a finite time interval. The specialized efficiency of MPC in the management of bioreactor pH is because of the ability to handle nonlinearity and constraints in acid base control of

neutralization reactions in the bioreactor. MPC is an optimization problem which solves at time interval of controlling on the basis of the existing values and prediction model in order to form an optimum series of control input. An example is that robust MPC techniques have been covered in [48] to robustly control a bioreactor, where in such case the pH variations is assumed to be a disturbance variable and variableness in the controller is predicated on the fact that the substrate level and the biomass level is maintained at optimum level.

There are several advantages of MPC on the bio process control. It can also deal with multivariable systems and input and output constraints can be imposed and this is of value in a complex bioreactor situation. It is predictive in that it can predict future disturbance and pre-compensate control actions in advance and this shall make the stabilization of PH to be smooth and steady. It is particularly effective in operations whose time lag is huge or where the overshoot must be prevented without damaging sensitive cultures of life. However, MPC has a limit to it. It requires a useful and up-to-date model of the process and that could be difficult to be found in biological systems as they are dynamic and non-linear. Real - time Applications In real-time, the optimization the MPC invokes must be done by computation, which can be a challenging scenario. In addition to this, performance of control can lead to an increase in stability and call to instability when the model becomes outdated or fail to capture some dynamics [48].

2.5.5. Neural network control

That is Neural Network Control when complex, nonlinear models, such as bioreactors, are modelled and controlled using artificial neural networks (ANNs). Neural networks have the advantage in pH regulation by being able to discover the nonlinear mapping between the acid/base flow rates and pH levels by being fed with only data (i.e., without an explicit mathematical model). It is particularly useful in bioprocesses, as systems dynamics are hard to model because of biological variability and variable behaviour with time. As an illustration, one can mention a work by Liu et al. that suggests a neural network-based PID controller on a process of pH neutralization. The system applied neural network to recognize the process dynamics and self-adjust the PID parameters in real time to better their control performance with nonlinear conditions [44].

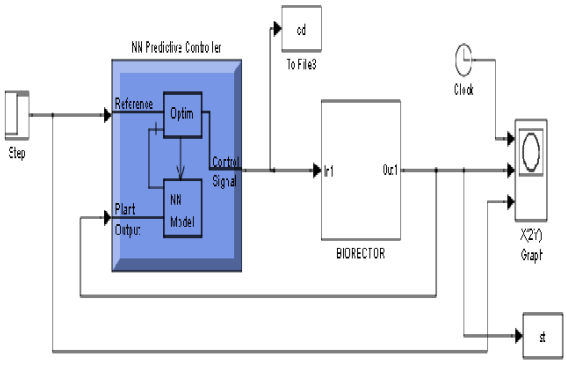

Fig. 9 Structure of a neural network representation of a predictive controller in block diagram form [49]

In a bioreactor, there are a number of benefits of using neural network control. It can be very efficient in capturing the nonlinear and time varying behaviour of the pH dynamics which is otherwise ill suited to be controlled with the classic linear designs. Neural networks are robust to disturbances and model uncertainties, because they can be trained with either historical or simulated data that

generalizes to a large variability of operating conditions. With this flexibility, the stability and pH control are also increasing, which is important towards maximum biological activity [44]. But the control of the neural network has drawbacks as well. Neural networks are often difficult to train and need big datasets and tuning not to overfit the training set but also to be generalizable. The resultant models may be computationally demanding and are incomprehensible, which makes them difficult to justify and troublesome. Also, neural network controllers may lose their performance in the case when the system works beyond the extend in which the training data were valid or a biological process changes in an unforeseen manner [49]. For illustration fig. 9 present the structure of a neural network representation of a predictive controller in block diagram.

2.5.6. Statistical process control (SPC)

The Statistical Process Control (SPC) is the process tool-based method of quality management in which a process is monitored and controlled with the help of the statistical tools. The SPC is not being applied as a control method in the traditional feedback meaning to the bioreactor pH and rather it is being applied as a process monitoring tool which makes conditions such that the process does not deviate out of process range. It means the usage of control charts, the ability of the processes, and the monitoring of trends, to identify the deviations of the normal conditions of work. Through SPC on trend monitoring of pH, this enables to detect the symptoms of imminent drift or instability system early enough and to take necessary action before the process has gone beyond control. The fig. 10 explain the sequences of the steps for statistical process control. Bioprocessing can also deploy the SPC with the help of feedback system in the upstream processing in order to augment the reliability, and it offers regulatory conformity [50].

It provides a structured manner in which one should observe the stability of a process as well as how to monitor anomalies that are required to bring stability to processes to attain the same quality of the product. The trend and variation in the pH value can also be observed through SPC and this has the scope of early intervention to remove the likelihood of failed or spoilt batch. It also makes improvement continuous since it can determine where the variability of the process can be diverted [50]. SPC however possesses its inadequacies in performing dynamic controlling activities. It is reactive in nature rather than being proactive i.e., it does not directly make a change in the control inputs instead it relies upon human or computers intervention after a deviation is noticeable. In addition, SPC also assumes that the process is in the state of statistical control, which is not the case when the process involves highly variable biological systems. The technology of SPC is, thus, best suited when applied along with active control like PID or MPC and not as a standalone one [50].

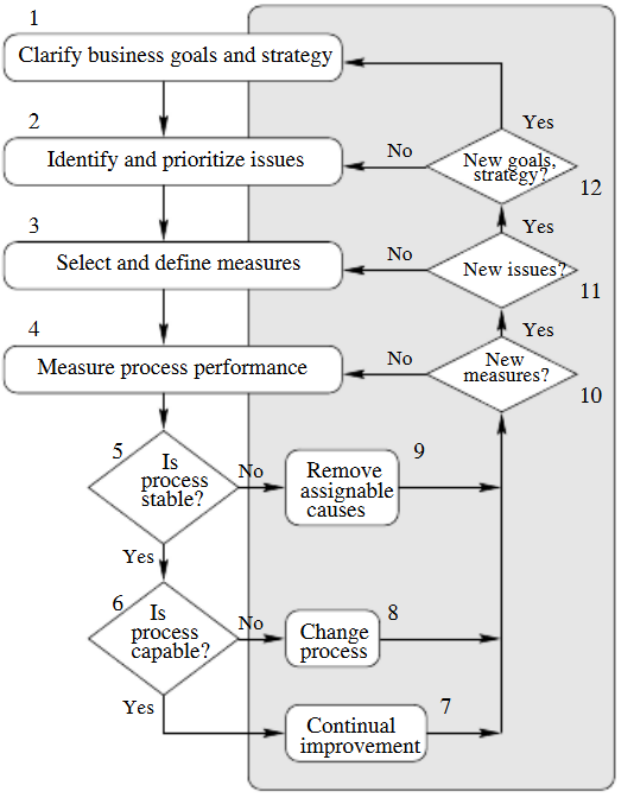

Fig. 10 Statistical Process Control sequence of steps [51]

2.5.7. Model free adaptive control (MFAC)

Model free adaptive control limits reliance on a precise, mathematically denoted description of the process. Rather than keeping the control actions fixed, it uses real time input-output data. The MFAC has particular appeal compared to approaches based on PID in pH control of bioreactors process where MFAC is flexible to handle high nonlinearity and variability in biological processes without the need of complex system identification/modelling or time-consuming tuning conditions. Especially, a variant of the MFAC-SA-PID is suggested; in the vicinity of the neutralization point, the MFAC is used with enhanced control in a nonlinear and sensitive region [52]. Under this approach, the controller could also communicate with the process dynamics through only observed information which is consequently as a result, very realizable and adaptable.

There are diverse advantages of employing the MFAC in the bioprocess regulation. It also relieves the development of the controllers because they do not need the detailed modeling of the process. This aids the applicability of MFAC especially where the parameters of systems had changed with time or other areas that could not be measured. It is also backed by its computational strength and in case of the actual implementation it is efficiency can already be achieved in real-time with minimal adjustment necessary and it is applicable in embedded systems and the industrial realm [52]. Its performance relies most on the quality and the frequency of the input-output data or it can fail to perform in case of large measurement noise or delay. Besides, despite the flexibility of MFAC to adjust to any form of change, MFAC is not particularly suitable in a situation that is highly constrained or in multivariable system where predictions have to be made. In spite of these challenges, MFAC

remains a useful option when aiming at enhanced and flexible pH regulation within bioreactors especially in a scenario when the flexibility and complexity are to be preferred [53].

3. Process Description and Mathematical Model of The Process

The investigated system is a fed-batch biotechnological process shown in fig. 11 which is carried out in a stirred tank bioreactor. The process configuration matches the experimental setup of [1], where microbial growth results in the generation or consumption of hydrogen ions, in turn influencing the pH of the medium in which the microorganism is grown.

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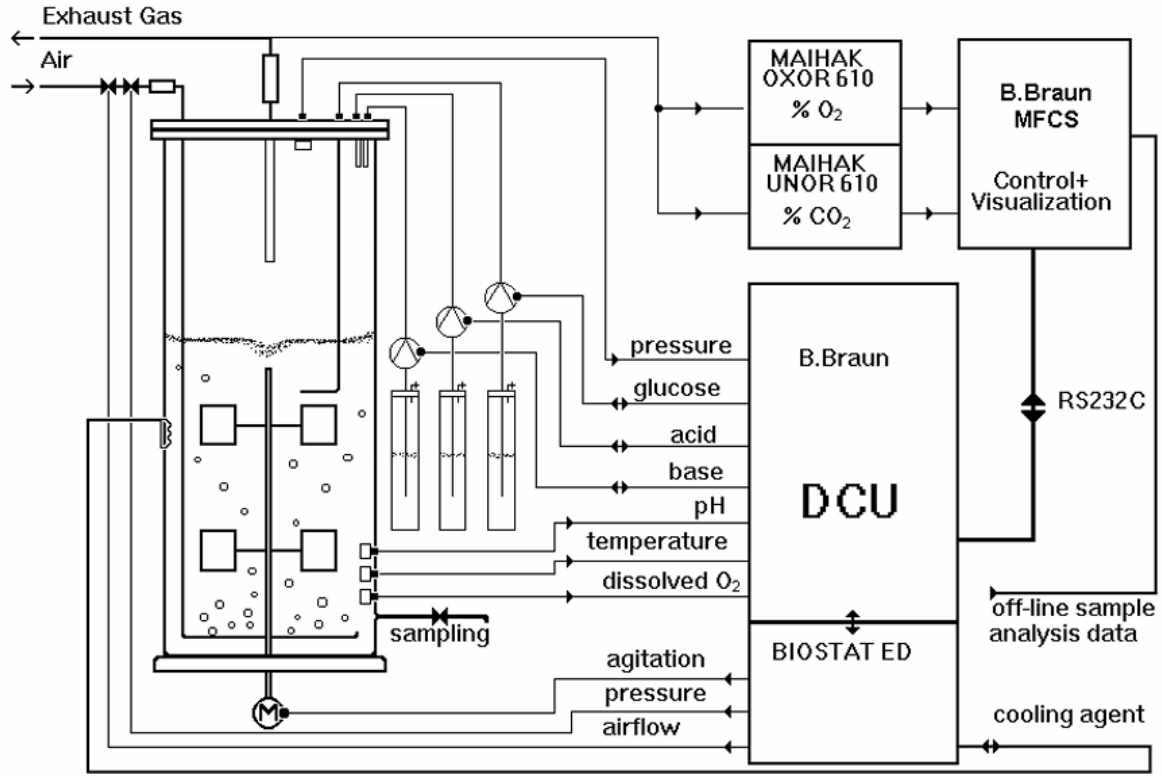

Fig. 11 Experimental Setup [1]

3.1.1. Process Overview

The investigated system is a fed batch biotechnological cultivation process conducted in a stirred tank bioreactor. In the fed-batch operation the nutrients are added continuously or intermittent during the cultivation and therefore higher concentration of biomass and better product yields can be achieved compared to batch operation. However, this way of operation also causes extra complexity in process dynamics because of a continuously changing volume and concentration of the substrate [1].

The bioreactor is considered as well mixed, so that there is no uneven distribution of biomass, nutrients and dissolved gases in the cultivation medium. The process includes continuous aeration which enables aerobic microbial growth and allows the estimation of metabolic activity by exhaust gas analysis [1].

3.1.2. Controlled and Manipulated Variables

The controlled variable is the pH of the cultivation medium. Maintaining the pH is of vital importance, as even a short-term deviation can have a significant influence on the microbial sustainability and productivity.

Manipulated Variable: The flow rate of the alkali solution which are added to neutralize the hydrogen ions produced by metabolic reactions. Acid addition is not taken into account in this work as in the reference paper [1] modeling approach.

3.1.3. Disturbances and Process Challenges

The dominating disturbance which influences the pH dynamics is biomass growth. As the concentration of biomass increases the rate of metabolic activity increases and hence the production rate of hydrogen ion H+ increases. This disturbance is highly time-varying, and not easily measured directly online, which makes the controller design difficult. Additional challenges include [1]:

- nonlinear titration of the cultivation medium,
- time-varying reactor volume caused by feeding and addition of alkali, and
- delays and dynamics of pH measuring sensors.

These characteristics are the motivation for the use of advanced adaptive control strategies.

3.1.4. Process Assumptions

The following assumptions are made in the modeling and simulation:

1. Perfect mixing of the cultivation medium.
2. Uniform temperature in reactor.
3. Availability of online measurement for pH and dissolved oxygen.
4. Oxygen uptake rate (OUR) can be estimated by gas analysis.
5. Although acid and alkali dosing systems exist in the experimental setup, the pH control strategy and the mathematical model only take into account the addition of alkali and assume that pH disturbances are mainly due to the biological acid production. [1]

3.2. Mathematical Model of the Biotechnological Process

The mathematical model used in this work is derived from first principles and follows the formulation presented in [1].

3.2.1. pH and Hydrogen Ion Dynamics

The main controlled variable in the analysed control system is pH level of the medium which is related to the concentration of free hydrogen ions as follows [1]:

$$\mathrm{pH} = -\log_{10}(C_{H+}) \tag{4}$$

The dynamic balance of hydrogen ions in the fed batch reactor is given by:

$$\frac{dC_{H^+}}{dt} = \alpha_1 \mu x + \alpha_2 x + \frac{F_{pH}}{V}\left(C^0_{H^+} - C_{H^+}\right) - \frac{F_s}{V} C_{H^+} \tag{5}$$

where:

- (C_{H+}) is the hydrogen ion concentration,
- (x) is the biomass concentration,
- (μ) is the specific growth rate,
- (V) is the reactor volume,
- (FpH) is the alkali flow rate,
- (Fs) is the feed flow rate.

This equation takes into account the production of hydrogen ions by metabolic activity, neutralization by addition of alkali, and dilution by feeding.

3.2.2. Biomass Growth Model

Biomass growth in the fed-batch process is modelled by:

$$\frac{dx}{dt} = \mu x - \frac{F_s + F_{pH}}{V} x \quad (6)$$

The initial concentration of biomass is set at the start of the cultivation process.

3.2.3. Oxygen Uptake Rate (OUR)

The oxygen uptake rate is related to biomass growth and maintenance:

$$\mathrm{OUR} = \beta_1 \mu x V + \beta_2 x V \quad (7)$$

3.2.4. Volume, Feed, and Alkali Flow Dynamics

The volume of the reactor is varied by feed and addition of alkali:

$$\frac{dV}{dt} = F_s + F_{\mathrm{pH}} \quad (8)$$

$$F_s = \frac{\mu x V}{Y_{xS} S_0} \quad (9)$$

$$F_{pH} = Y_{ax} \mu x V \quad (10)$$

Feed and alkali flows are related to biomass growth according to yield coefficients, as presented in the reference paper.

3.2.5. Model Parameters and Initial Conditions

Model parameters (α_1, α_2, β_1, β_2) are adopted based on identified values reported in literature. Initial conditions for $C_{H+}0$, pH, biomass concentration and volume are chosen to be typical experimental conditions. The model parameter values and initial conditions are shown in fig. 12.

Table 3: Model Parameters and Initial Conditions [1]

Model parameter	Value		Units
	Phase 1	Phase 2	
α_1	$0.422 \cdot 10^{-7}$	$0.4088 \cdot 10^{-7}$	[mol/g]
α_2	$0.011 \cdot 10^{-7}$	$0.0179 \cdot 10^{-7}$	[mol/(gh)]
C^0_{H+}	$-5.367 \cdot 10^{-5}$		[mol/l]
β_1	0.8646	1.4700	[g/g]
β_2	0.0180	0.0038	[g/(gh)]

4. Model-Free Adaptive (MFA) Controller

Model-Free Adaptive (MFA) control, as its name suggests, is an adaptive control method that does not require process models. A Model-Free Adaptive control system is defined to have the following properties: Precise quantitative knowledge of the process is not necessary; Process identification mechanism or identifier is not included in the system; Controller design for a specific process is not needed; Manual tuning of controller parameters is not required; and Closed-loop system stability analysis and criteria are available to guarantee the system stability Variations of the core MFA control technology address specific control problems as described here [2,6].

1. SISO (Single Input Single Output) MFA to replace PID (Proportional, Integral, Derivative) controller so that manual controller tuning is eliminated,
2. Nonlinear MFA to control nonlinear processes,
3. MFA pH controller to control pH processes,
4. Feed forward MFA controller to deal with measurable disturbances,
5. Anti-delay MFA to control processes with large time delays,
6. Robust MFA to protect the process variable from running outside a bound,
7. Time-varying MFA controller to control time varying processes,
8. Anti-delay MFA pH controller for pH processes with varying time delays, and
9. MIMO (Multi Input Multi Output) MFA to control multivariable processes. [6]

4.1.1. Motivation for MFA Algorithm based pH Control

The nonlinear and time-varying nature of the pH process limits the performance of both fixed and adaptive gain scheduled controllers. Model-Free Adaptive control offers another form of control that does not require the use of an explicit process model and adapts directly based on control error.

4.1.2. SINGLE-LOOP (SISO) MFA CONTROL SYSTEM

Fig. 13 shows an example of a single loop MFA control system containing a single input, single output (SISO) process, one SISO MFA controller, and a feedback loop. The control objective is for the controller to generate an output u(t) to make the process variable y(t) track the given trajectory of its setpoint r(t) in the face of variations of setpoint, disturbance and process dynamics [2]. This means that the task for the MFA controller is to minimize in an on-line fashion the error e(t), where e(t) is the difference between the setpoint r(t) and the process variable y(t) [2]. The minimization of error e(t) is achieved by

i. the regulatory control capability of MFA controller, and
ii. the adjustment of the MFA controller weighting factors that allow the controller to deal with the dynamic changes, disturbances, and other uncertainties of the control system [2].

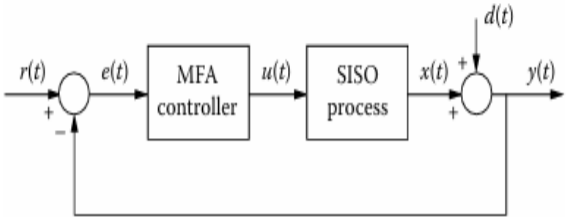

Fig. 12 General structure of the MFA control system [2]

4.2. MFA Controller Architecture

Fig. 14 shows the basic structure of one of the single-input single-output SISO MFA controllers. A multilayer perceptron (MLP) artificial neural network (ANN) is used in the design of the controller [2]. The ANN is having one input layer, one hidden layer containing N neurons, and one output layer containing one neuron. Within the neural network there is a group of weighting factors (wij and hi) that can be updated as is needed to vary the behaviour of the dynamic block. The algorithm for the updating of the weighting factors is based on the goal of minimum error between the setpoint and process variable. Since this effort is identical with the control objective, the adaptation of the weighting factors can help the controller to minimize the error during the time that process dynamics are changing. Also, from another point of view, the artificial neural network based, MFA controller 'remembers' a part of the process data with which valuable information for the process dynamics can be gained [6]. In comparison, a digital version of the PID controller stores the current, and the last two samples. In this respect, PID has no memory (almost) and MFA has the memory that is necessary to a "smart" controller [2,6].

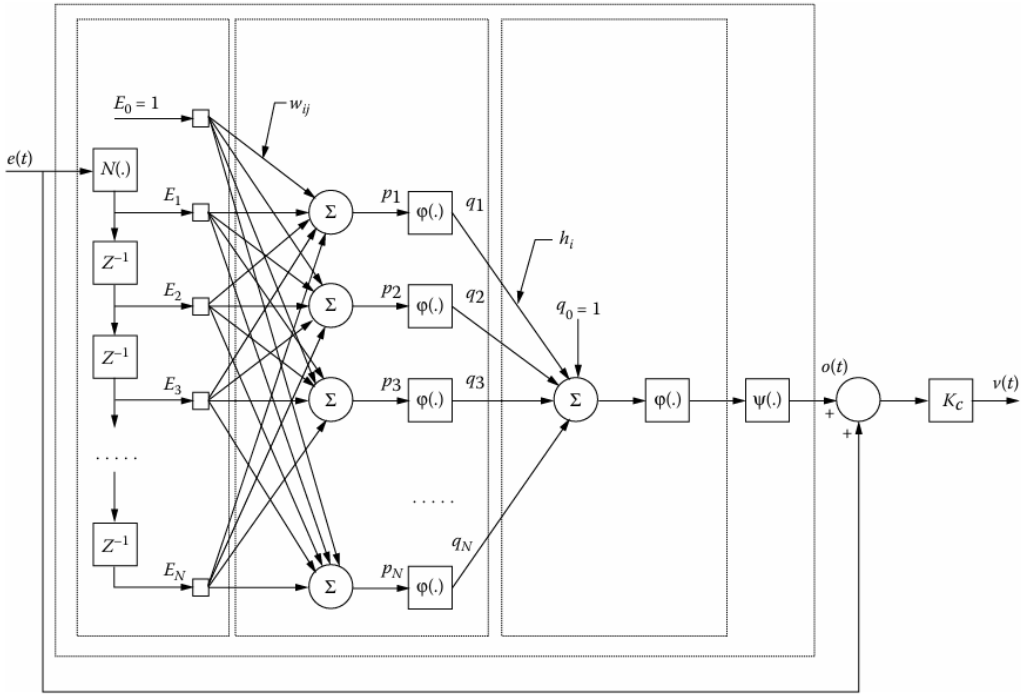

Fig. 13 Architecture of a SISO MFA controller [2]

4.3. SISO MFA Mathematical Control Algorithm

The core MFA control algorithm comprises the following difference equations:

i. Weighted sum with bias

$$p_j(n) = \sum_{i=1}^{N} w_{ij}(n)\ E_i(n) + 1 \qquad (11)$$

ii. Activation of hidden unit

$$q_j(n) = \phi\left(p_j(n)\right), \quad (12)$$

iii. Output mapping with nested activations

$$o(n) = \psi\left[\phi\left(\sum_{j=1}^{N} h_j(n)\ q_j(n) + 1\right)\right] \quad (13a)$$

$$= \sum_{j=1}^{N} h_j(n)\ q_j(n) + 1, \quad (13b)$$

iv. Control law combining output and error

$$v(t) = K_c\,[\,o(t) + e(t)\,] \quad (14)$$

v. Weight update rule

$$\Delta w_{ij}(n) = \eta\, K_c\, e(n)\, q_j(n)\left(1 - q_j(n)\right) E_i(n) \sum_{k=1}^{N} h_k(n) \quad (15)$$

vi. Hidden-to-output weight update

$$\Delta h_j(n) = \eta\, K_c\, e(n)\, q_j(n) \quad (16)$$

where n denotes the nth iteration, $o(t)$ is the continuous function of $o(n), v(t)$ is the output of the MFA controller, $Kc > 0$ and is the MFA controller gain, and wij and hj are weighting factors. The weighting factors can be updated online at every sample interval using the following formulas [2].

5. MATLAB Simulink Implementation of the Process Model

The full process model was modelled in MATLAB Simulink. Each major physical phenomenon was modelled by a separate subsystem so that the flow of signals could be seen clearly and could be easily changed.

5.1.1. MATLAB Simulink Model Architecture

The designed Simulink model shown in fig. 15 consists of interconnected subsystems representing:

- hydrogen ion dynamics,
- biomass growth,
- oxygen uptake estimation,
- volume dynamics.

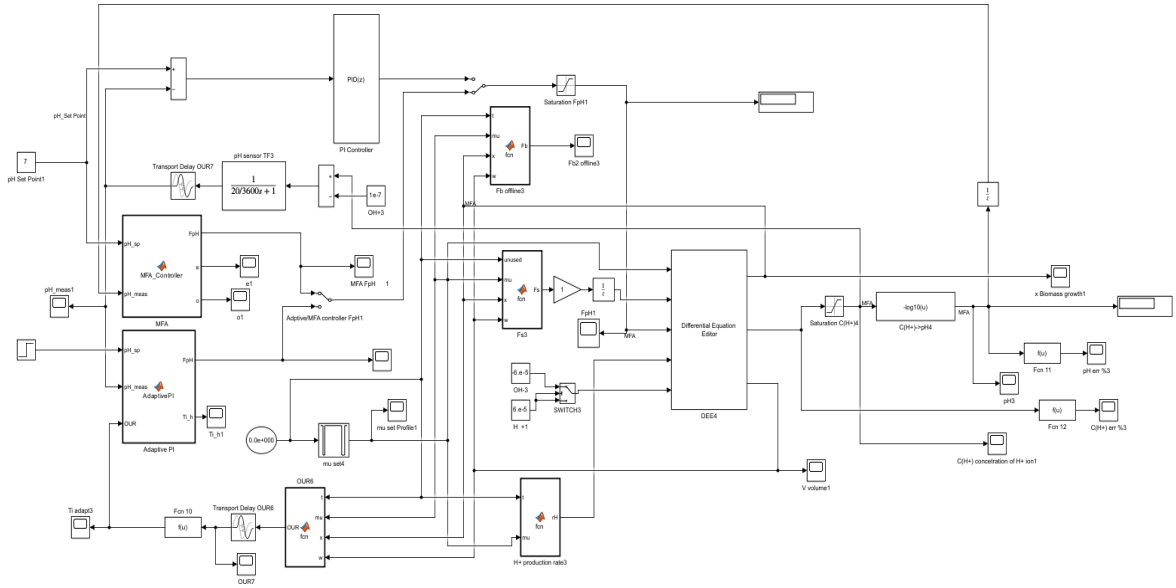

Fig. 14 Simulink Model

5.1.2. Simulation Settings

- Solver: Variable step discrete solver ode45
- Sample time: 0.001h or 3.6s
- Total simulation time: 10 hours

5.2. Baseline Controllers for Comparison

A classical fixed parameter PI controller was implemented as baseline. For nominal operating conditions controller parameters were adjusted. This controller is just a reference to illustrate the limitations of any fixed parameter control in a nonlinear and time varying process.

For adaptive strategy, the gain scheduled PI controller shown in fig. 16 was implemented exactly as described by [1]. The controller uses gain scheduling to adapt the integral time parameter based on the oxygen uptake rate.

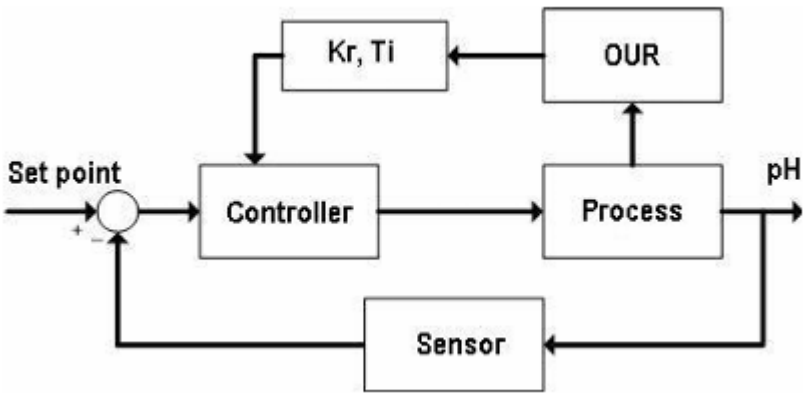

Fig. 15 General structure of the gain-scheduled PI control system [1]

$$T_i = \frac{\gamma_1}{\mathrm{OUR} + \gamma_2} \tag{10}$$

An optional gain scheduling rule for the proportional gain is:

$$K_r = K_{r0}\,\frac{V}{V_0} \tag{11}$$

No modifications were made to the original control structure. The simple PI and gain-schedule PI controller is used solely as a benchmark for performance comparison with MFA.

5.3. Simulation Results with MFA Control

The MFA controller was implemented as a separate Simulink subsystem using MATLAB Function and connected to the same process model used for the PI and adaptive PI controllers.

5.3.1. Simulink Result of pH, FpH, x, C(H+)

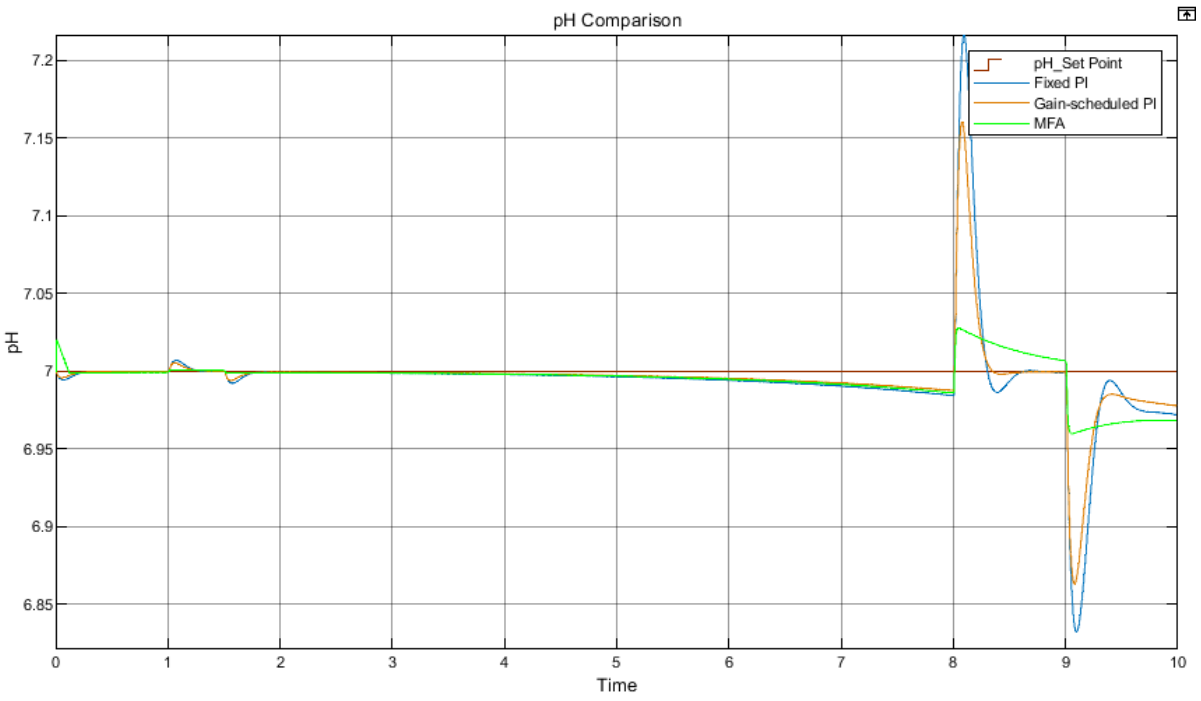

Fig. 16 pH Comparison Graph

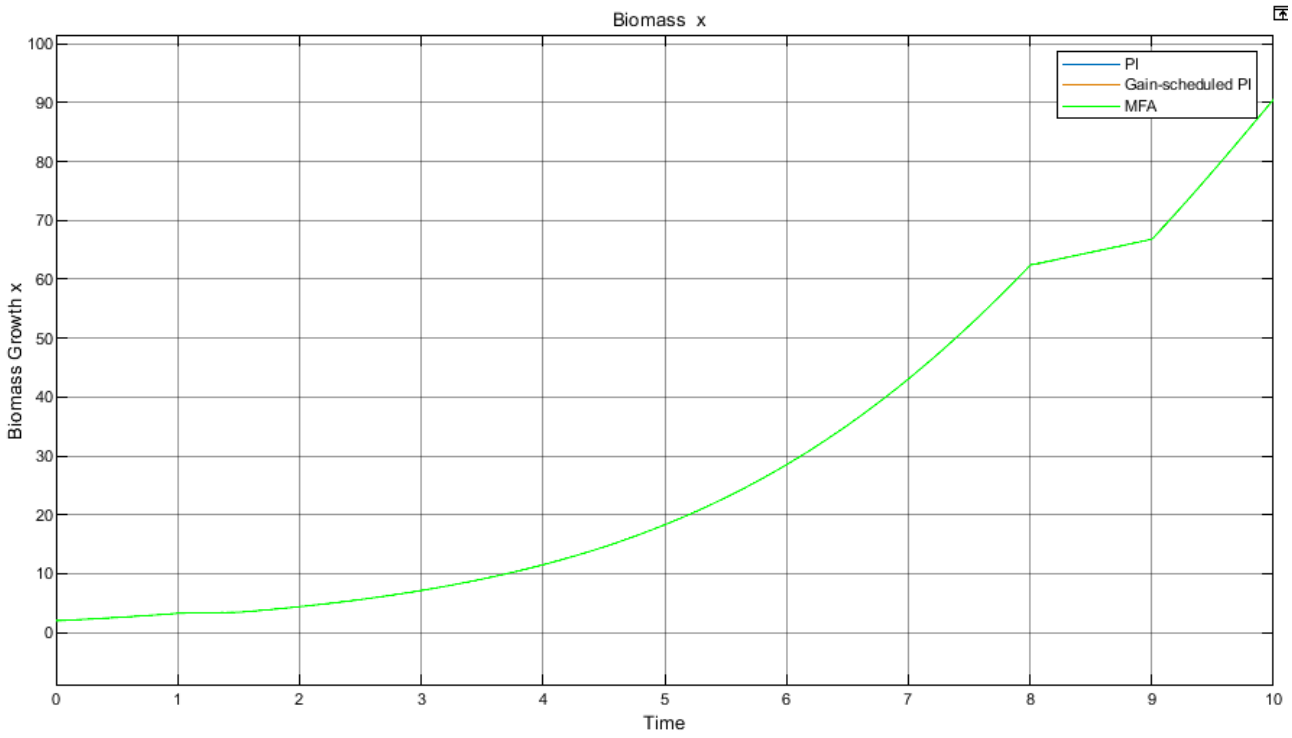

Fig. 17 Biomass concentration x (g/L)

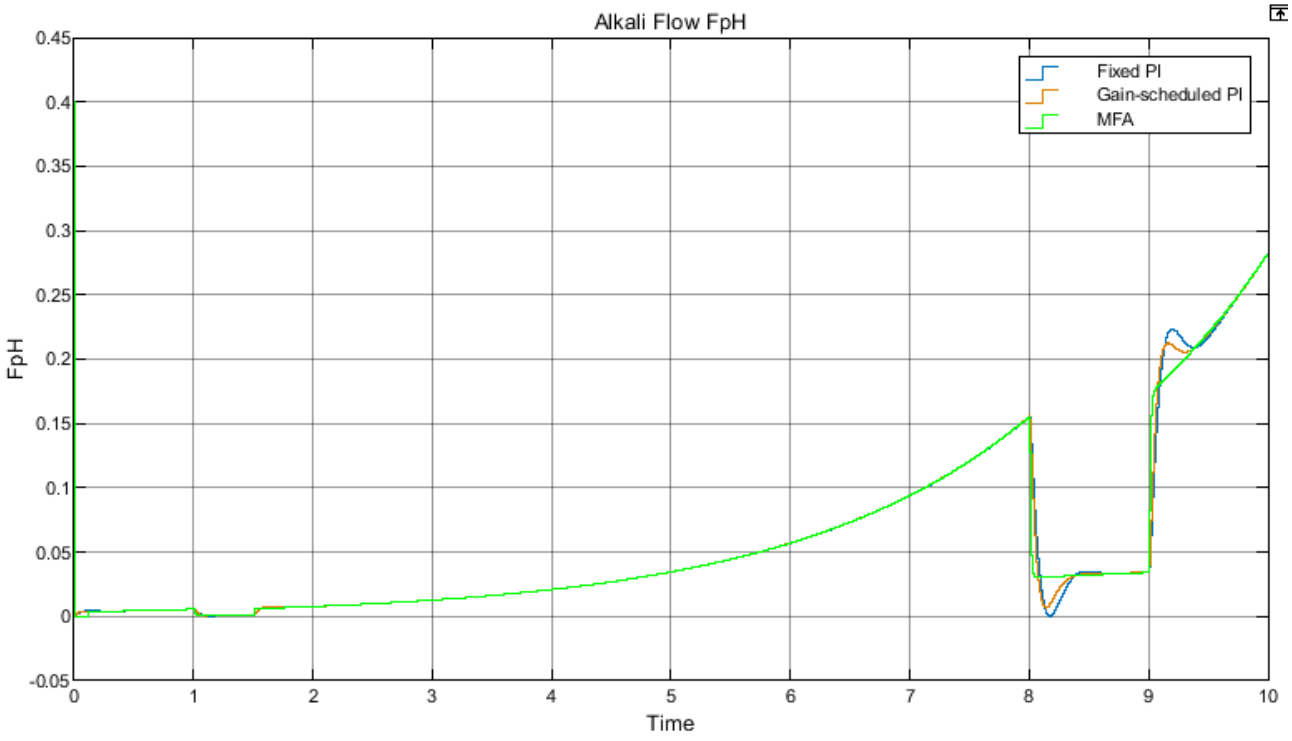

Fig. 18 Alkali Flow FpH (L/h)

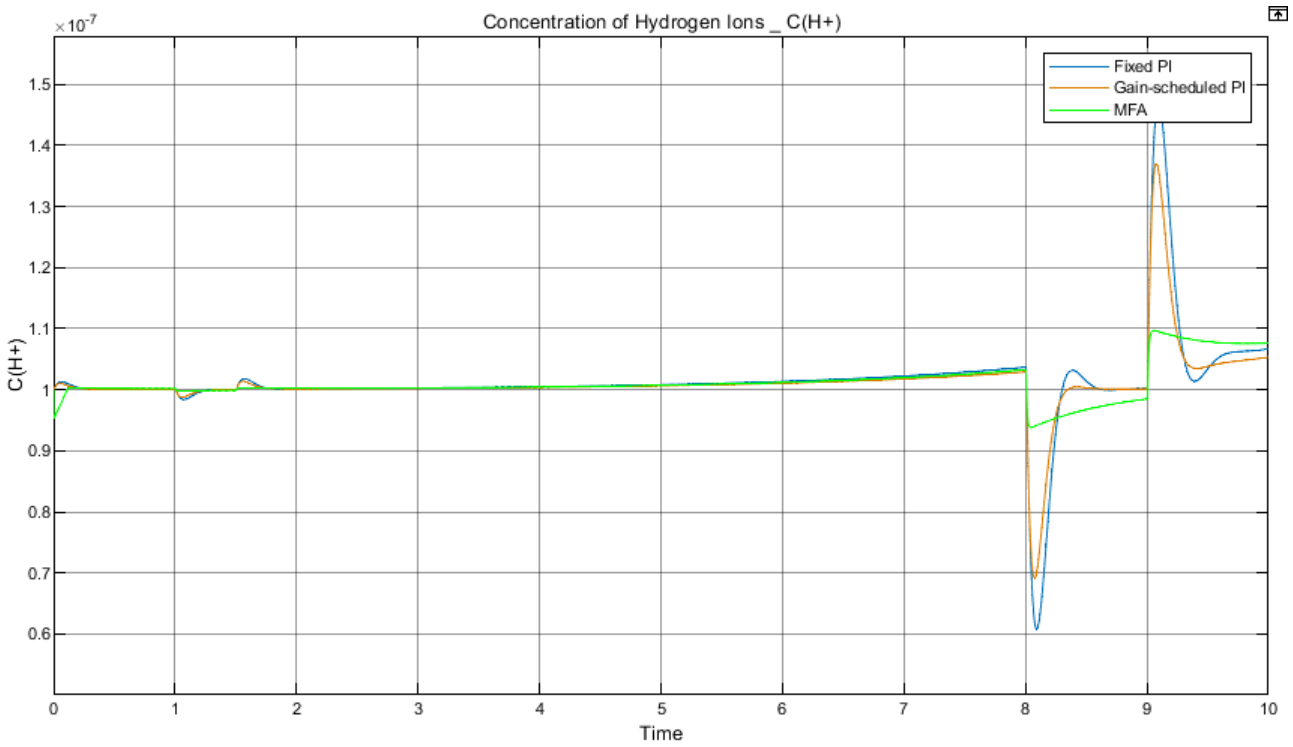

Fig. 19 Concentration of Hydrogen Ions C(H+) (mol/L)

5.3.2. MFA Performance Based on Different Learning Rate ETA

As the other plots (fixed, gain-sched, are the same for all three plots, I would make only one plot containing fixed, gain-sched., and several cased of MFA under different eta)

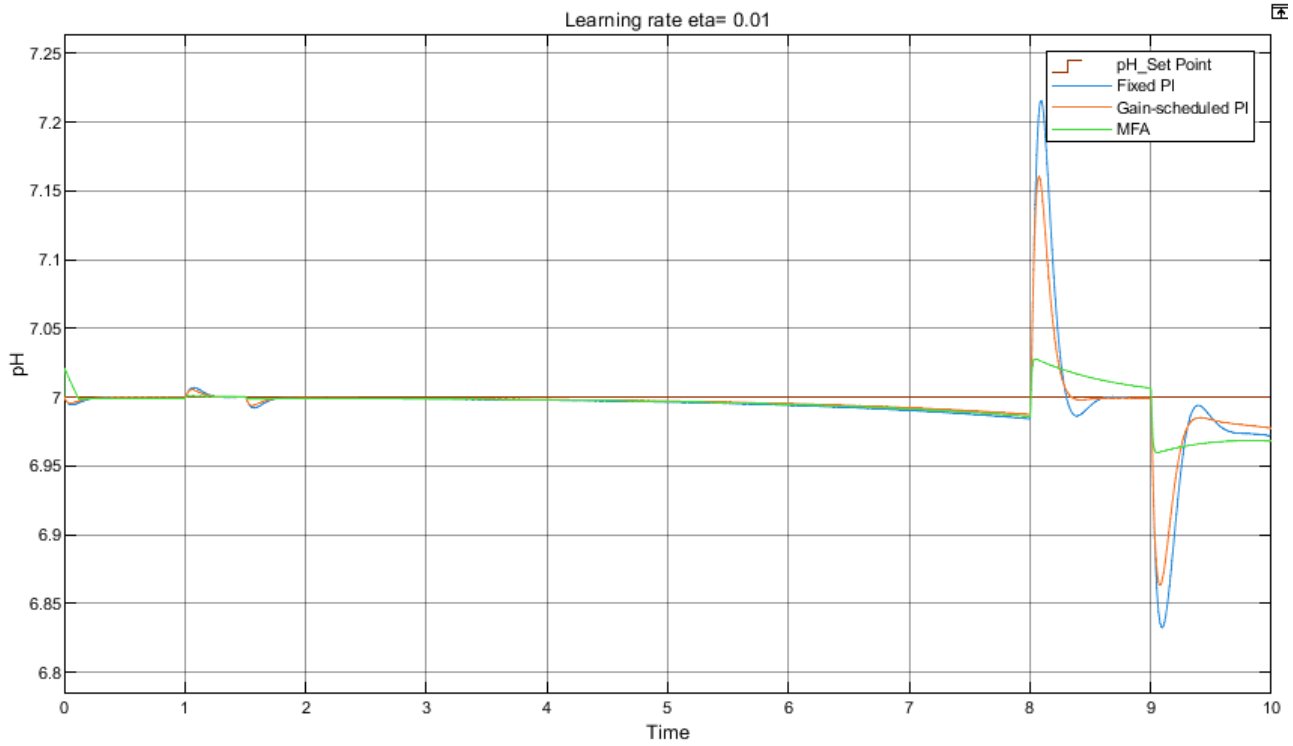

Fig. 20 The plot shows pH evolution under learning rate eta= 0.01

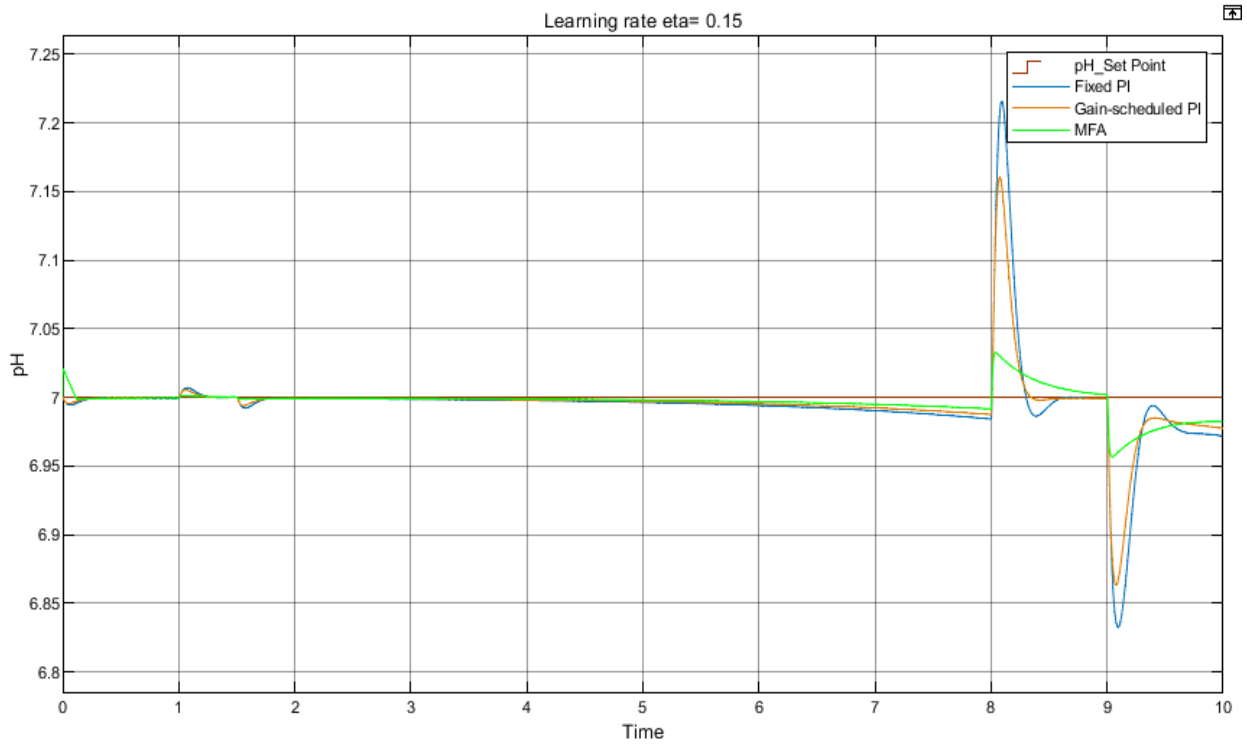

Fig. 21 The plot shows pH evolution under learning rate eta= 0.15

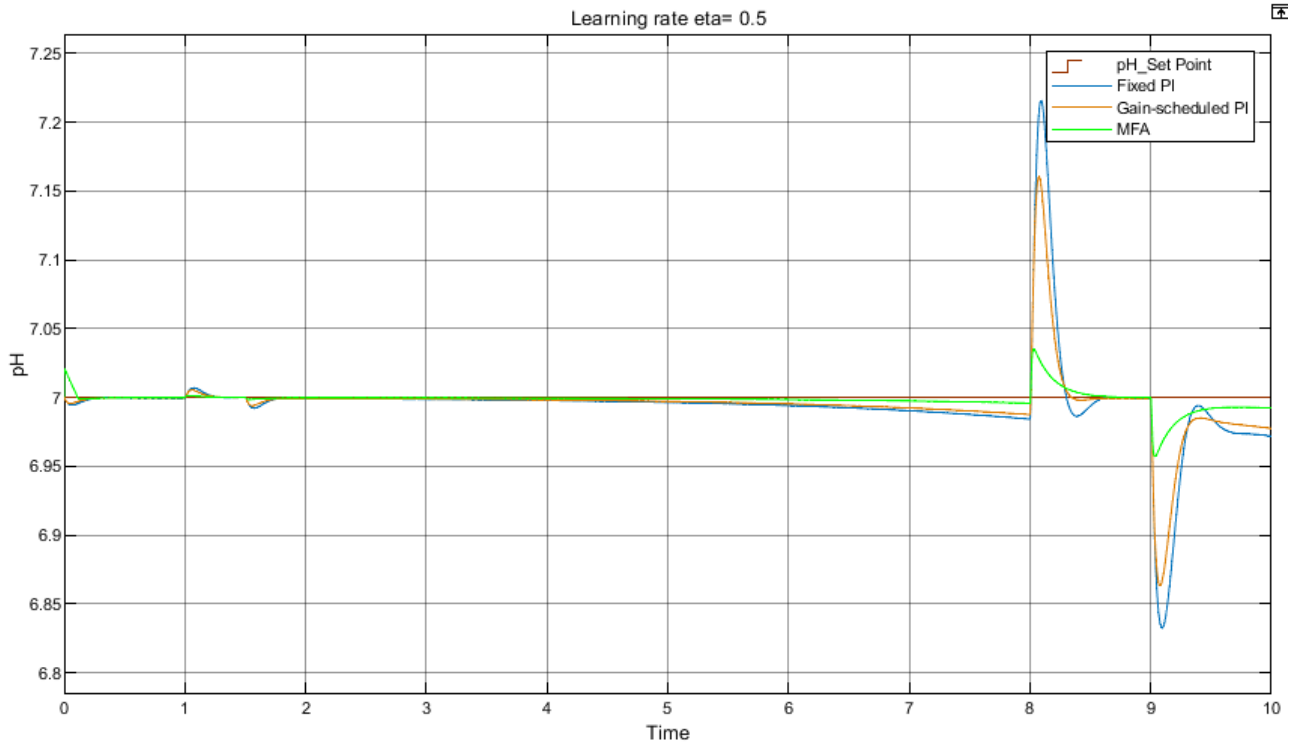

Fig. 22 The plot shows pH evolution under learning rate eta= 0.5

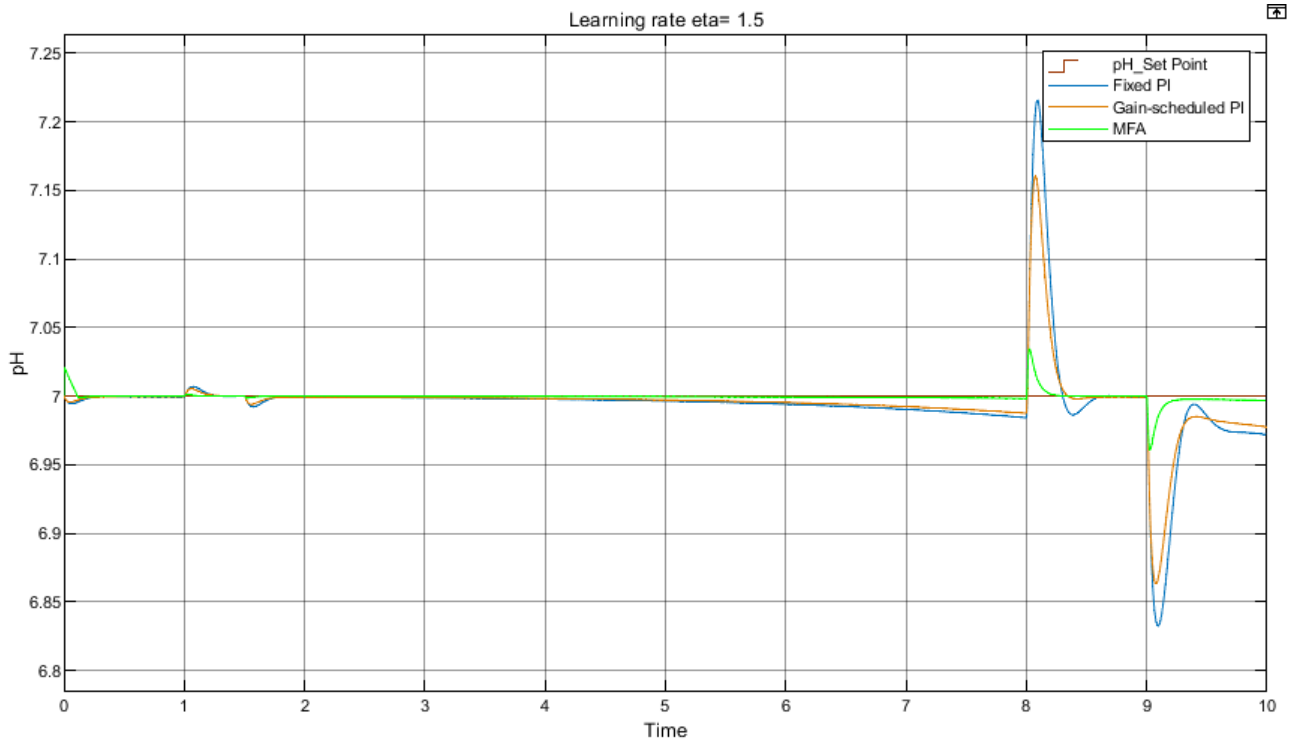

Fig. 23 The plot shows pH evolution under learning rate eta= 1.5

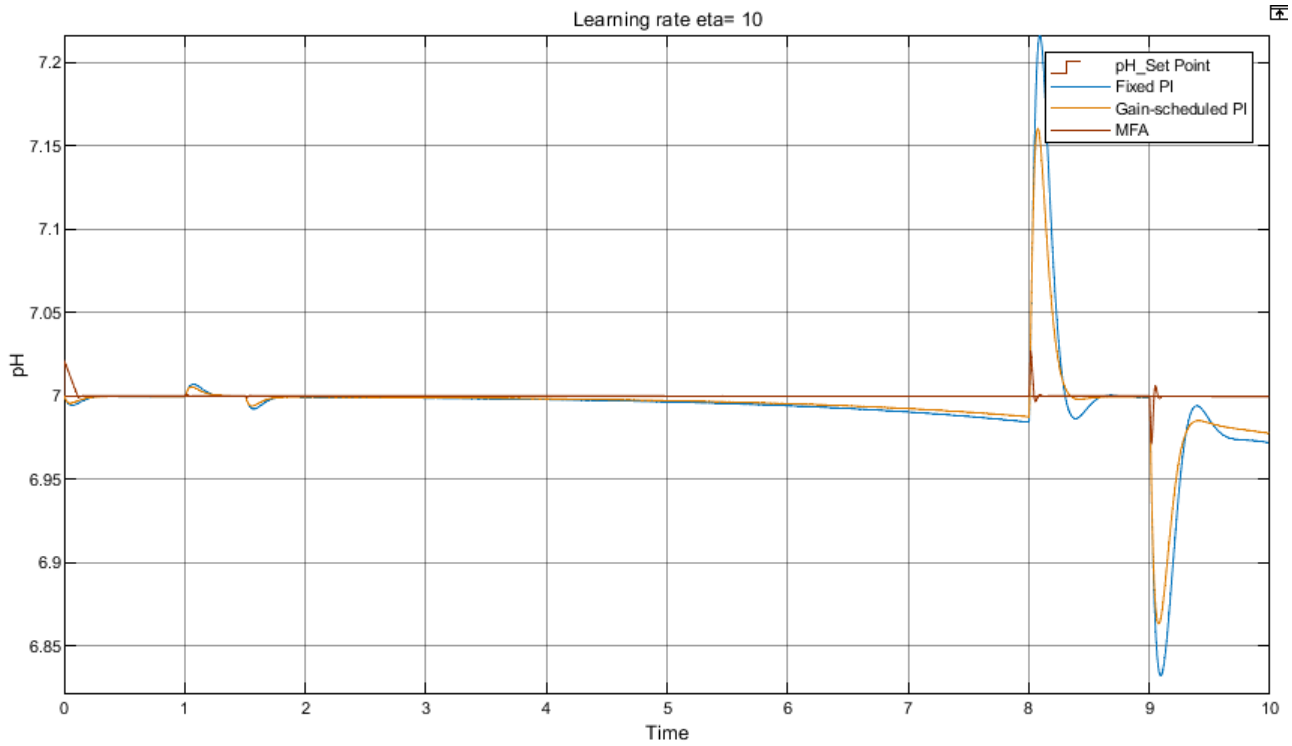

Fig. 24 The plot shows pH evolution under learning rate eta= 10

5.3.3. Setpoint Tracking and Disturbance Rejection Performance

Simulation results indicate the MFA controller is able to keep the pH near the target setpoint for the entire fed batch process. Certain disturbances in the form of changes in specific growth rate were

introduced. The MFA controller showed better robustness as compared to the PI and adaptive PI based controllers.

Controller	IAE	RMSE	Overshoot	Settling Time_h
PI	0.1147	0.031298	3.0861	0.152
Gain Schedule PI	0.087236	0.023284	2.292	8.1081
MFA	0.025969	0.0060352	0.50428	-

Fig. 25 MATLAB Results for setpoint tracking and disturbance rejection performance

5.3.4. Effect of Gaussian Noise on the Performance of Controller

To test the robustness of the control systems, gaussian noise was added to the measurements of the signal of the hydrogen-ion concentration. These included low noise, medium noise dominated scenarios, and highly noisy scenarios were considered.

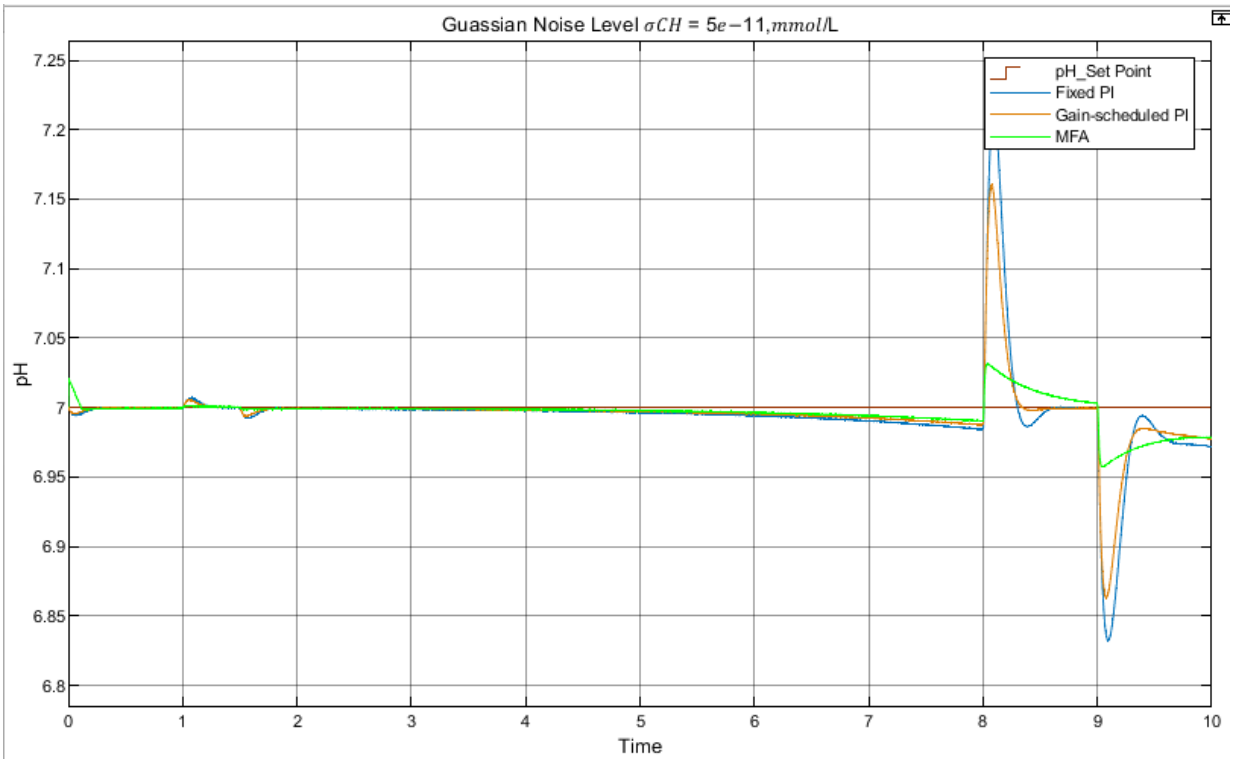

Fig. 26 Noise Intensity Level ($\sigma CH^{+} = 5e^{-11}$, $mmol$/L)

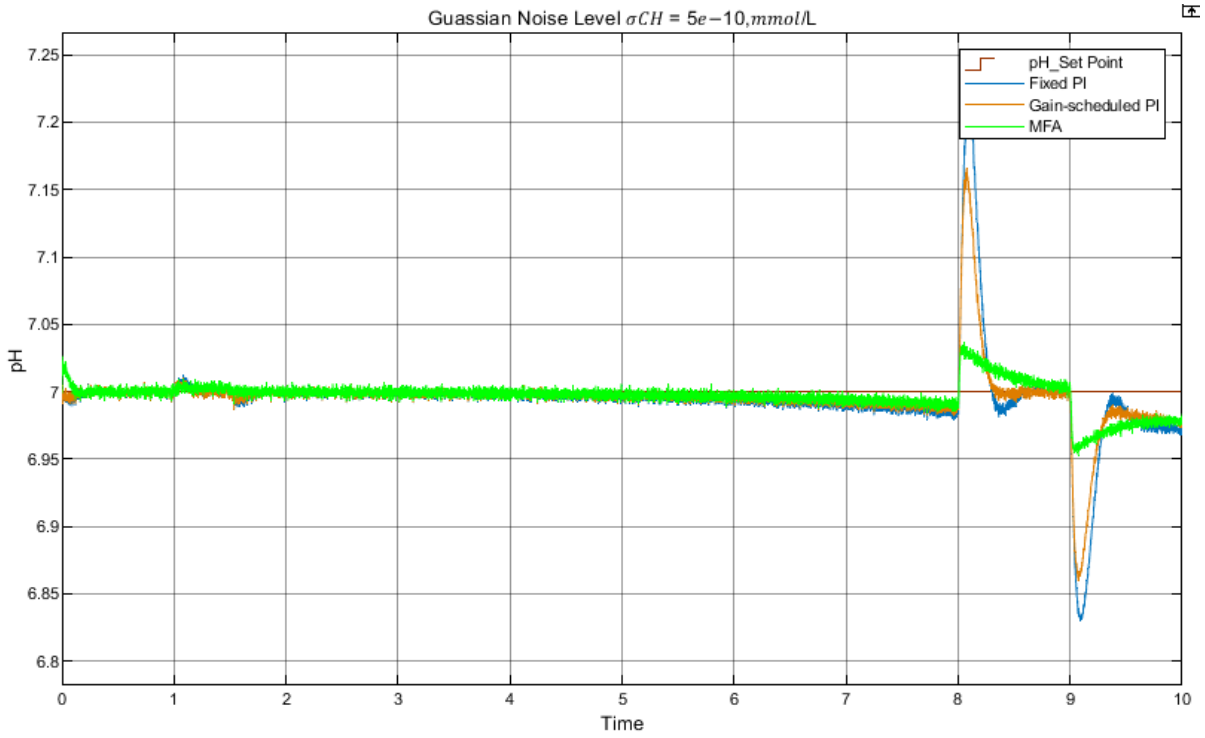

Fig. 27 Noise Intensity Level ($\sigma CH^{+} = 5e^{-10}$, $mmol$/L)

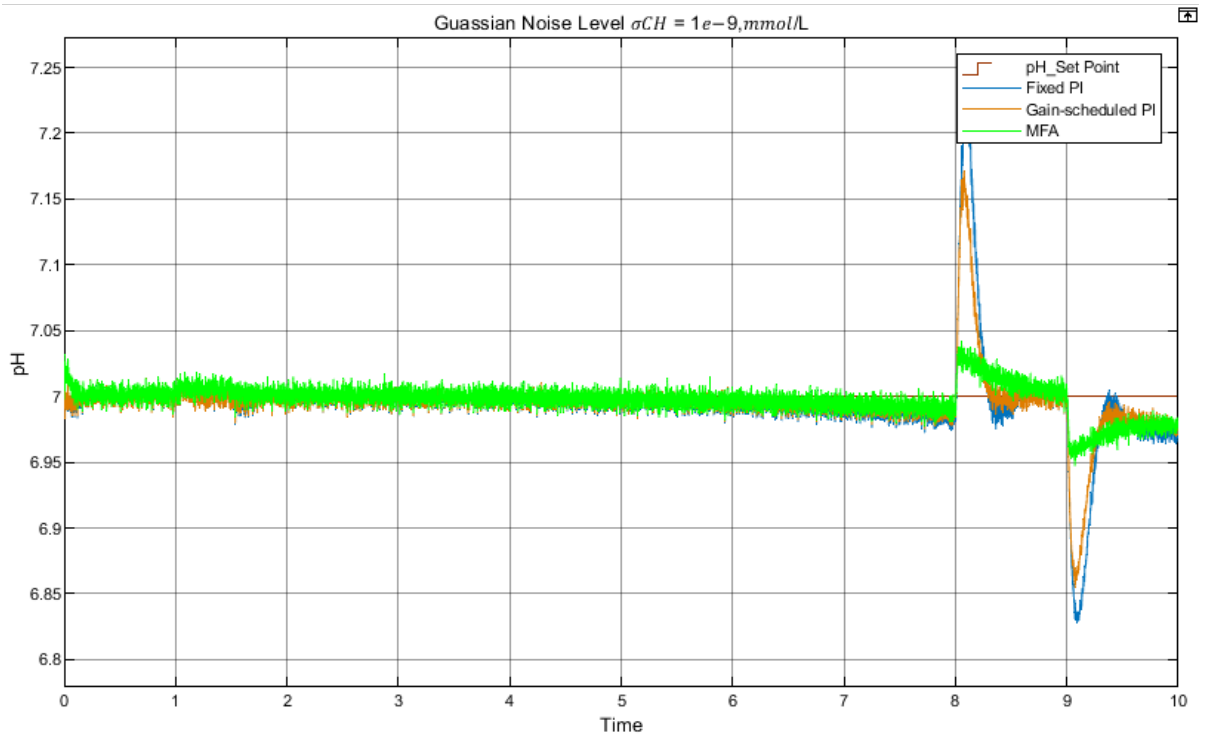

Fig. 28 Noise Intensity Level ($\sigma CH^{+} = 1e^{-9}$, $mmol$/L)

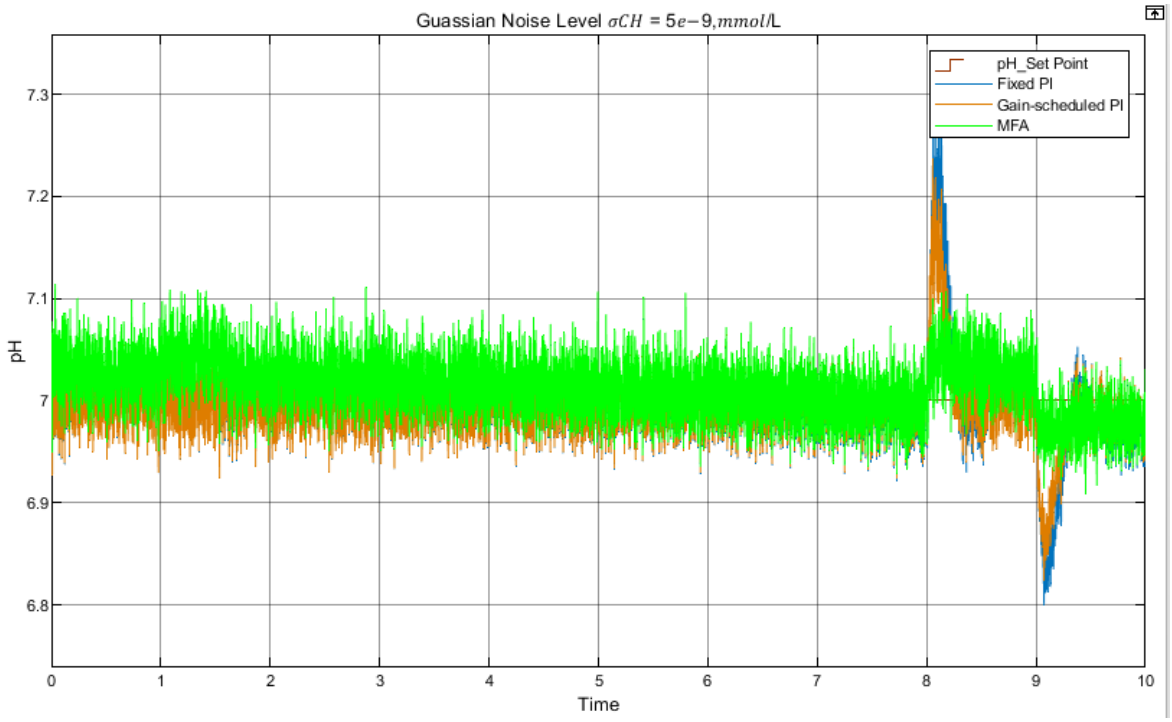

Fig. 29 Noise Intensity Level ($\sigma CH^{+} = 5e^{-9}$, $mmol$/L)

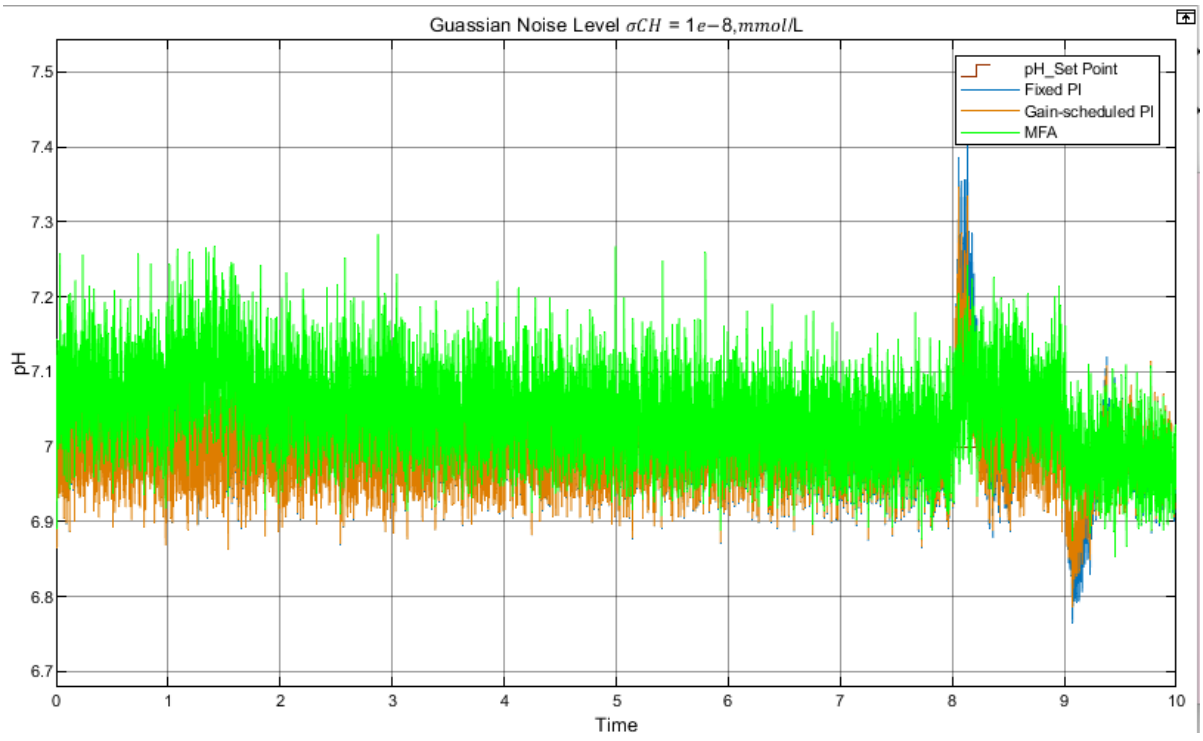

Fig. 30 Noise Intensity Level ($\sigma CH^{+} = 1e^{-8}$,$mmol$/L)

Noise	Controller	IAE	RMSE	Overshoot	Settling Time_h
5e-11	PI	0.27090	0.03822	3.491	9.105
	Gain schedule PI	0.21017	0.02885	2.614	8.129
	MFA	0.19460	0.02091	0.762	-
5e-10	PI	0.27205	0.03836	3.521	9.111
	Gain schedule PI	0.21130	0.02901	2.683	8.136
	MFA	0.19808	0.02134	0.826	-
1e-09	PI	0.27351	0.03864	3.601	9.111
	Gain schedule PI	0.21291	0.02934	2.761	8.136
	MFA	0.20639	0.02246	0.903	-
5e-09	PI	0.31300	0.04482	4.652	9.134
	Gain schedule PI	0.26632	0.03681	3.704	9.078
	MFA	0.27295	0.03756	1.934	-
1e-08	PI	0.43612	0.05959	6.955	9.380
	Gain schedule PI	0.40321	0.05343	5.274	9.134
	MFA	0.41021	0.05498	4.432	9.010

Fig. 31 Performance table of PI, Gain Schedule PI and MFA controller under the influence of gaussian noise

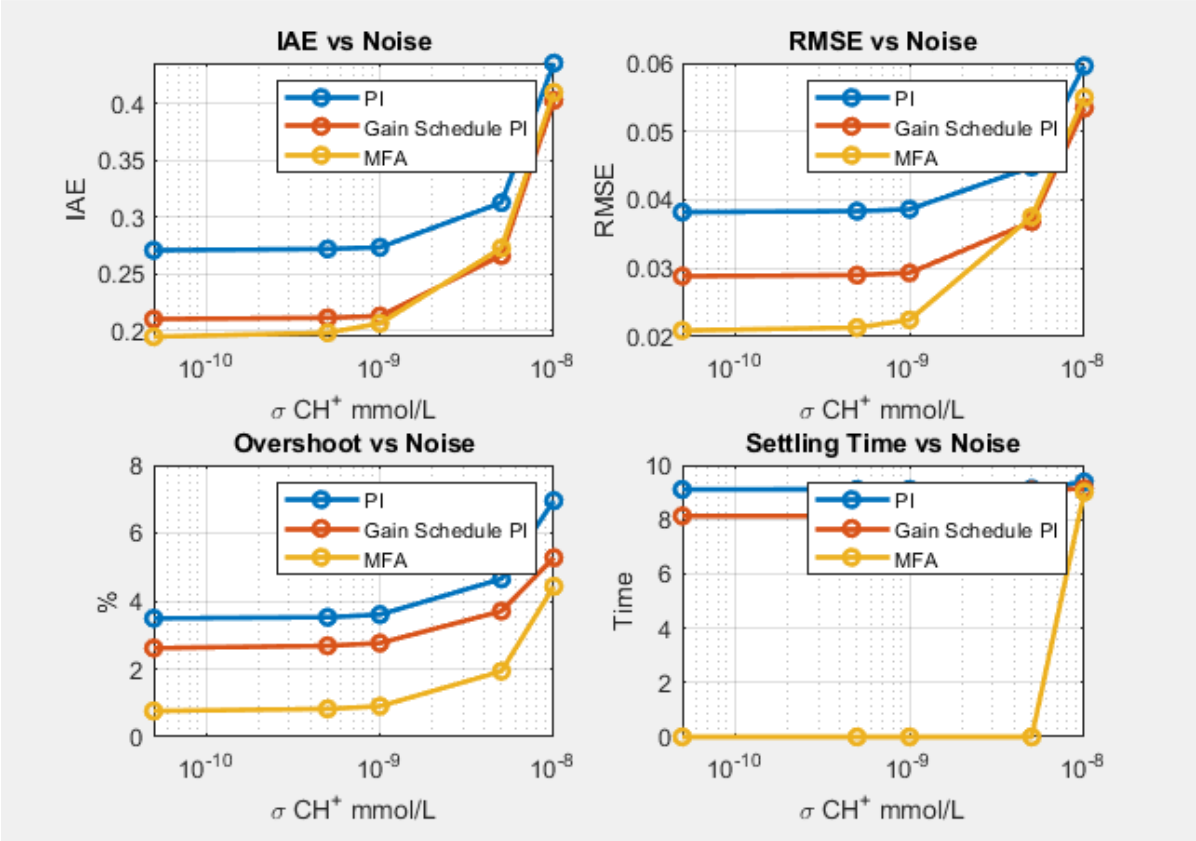

Fig. 32 Variation of IAE, RMSE, overshoot, and settling time for PI, gain-scheduled PI, and MFA controllers under different Gaussian noise levels.

6. Discussion of Results

The performance of the fixed PI, adaptive PI, and model-free adaptive (MFA) controllers were evaluated in case of pH regulation for a bioprocess over 10 hours of simulation time. The comparison was done in set-point tracking, disturbance rejection, control effort and robustness using both qualitative time-domain responses and quantitative performance index like IAE, RMSE, overshoot and settling time in a range of 2% pH tolerance band.

During nominal operation prior to the disturbance (0-8 h) all three of the controllers maintained the pH close to the set-point of 7.0. However, significant differences were found in the quality of tracking. The fixed PI controller had the highest transient deviations and small oscillations around the set-point, especially in the early stage of the batch. The adaptive PI controller improved the tracking performance by reducing amplitude of oscillation and steady state error. The MFA controller showed the tightest regulation and held the pH as close to the set-point with little deviation throughout the nominal period. This observed performance is quantified by the integral performance indices: the MFA controller showed the minimum IAE (0.02597), and RMSE (0.00604), which is notably better in comparison with both the fixed PI (IAE = 0.1147, RMSE = 0.0313) and gain schedule PI (IAE = 0.08724, RMSE = 0.02328). These results suggest that excellent tracking of set points is achieved by the MFA controller, which does not rely on an explicit process model.

The disturbance introduced at t=8h resulted in a significant deviation in pH and hydrogen ion concentration for all controllers, and thus a direct comparison of disturbance rejection capability could be made. The fixed PI controller had the greatest pH overshoot, which was at about 3.09% above the set-point, followed by the adaptive PI controller at 2.29%. In contrast, the MFA controller only allowed the overshoot to be 0.50%, which is a significant reduction in the peak deviation. This lower overshoot is also manifested in the concentration response of the hydrogen ion concentration, where the MFA controller was able to mitigate positive and negative spikes better than the PI-based controllers. The results show unambiguously that the MFA controller is more fluid and proportional to disturbance and does not exhibit aggressive corrective actions.

Settling time analysis was done with relative 2% pH tolerance band (6.86-7.14), which is consistent for the requirements of pH control in practical bioprocess. The fixed PI controller took around 9.15 h time to come back within the tolerance band after the disturbance and the adaptive PI controller took 8.11 h time to settle within the tolerance band. It is noteworthy that the MFA controller settling time is effectively zero according to choosen criteria, which means that the pH response was within the tolerance band for the entire disturbance period. However, a small transient is still present. This behaviour is very interesting to emphasize the superior robustness of the MFA controller and its capacity to absorb disturbances without exceeding the operational pH limits.
The control effort, which is represented by the alkali flow rate FpH, is further responsible for the observed differences in performance. The PI controller fixed on its output produced sharp changes in control input, especially after the disturbance that contributed to higher overshoot and longer recovery time. The adaptive PI controller was able to moderate the control action but still resulted in noticeable transients. In contrast, the MFA controller varied the alkali flow more smoothly and progressively with no abrupt changes and still effective pH regulation. This smooth control behaviour is one of the

main advantages of the model-free approach where the adaptation is done directly through error dynamics, but not on fixed or slowly varying gain structures.
The effect of the MFA learning rate was also explored. Lower learning rates led to slower adaptation and somewhat greater deviation after the disturbance whereas different values showed a decent balance between responsiveness and stability

Finally, the biomass growth profiles were almost identical for all three controllers, indicating that better control of pH achieved by the MFA controller does not have any adverse effects on the biological process. This ensures that the improved control performance has not come at the expense of productivity or process dynamics.

Overall, the results show that the MFA controller has better performance in both set-point tracking and disturbance rejection than fixed PI and adaptive PI controllers. The large decreases in IAE, RMSE, overshoot and settling time indicates the effectiveness of model free adaptive control strategy for nonlinear and time-varying bioprocess pH control

Effect of Gaussian Noise (σCH^{+})

Four performance criteria, namely, IAE, RMSE, overshoot (%), and settling time, were used to compare the performance of the PI, gain-scheduled PI, and model-free adaptive (MFA) controllers. The PI controller resulted IAE values of 0.271-0.274, RMSE values of 0.038-0.039 with overshooting of 3.491%-3.601% and settling time of approximately 9.11 at the lowest three operating levels (5×10-11 – 1×10-9). With the gain-scheduled PI controller the error indices were reduced to around 0.210 – 0.213 (IAE) and 0.028 – 0.029 (RMSE) while lower overshoot values of 2.614% - 2.761% and settling times around 8.13 were obtained. The MFA controller also reduced the error values, with IAE in between 0.194 and 0.206, RMSE in between 0.0209 and 0.0225, overshoot between 0.762% and 0.903%.

The PI controller had an IAE of 0.31300 and an RMSE of 0.04482 at the operating level of 5×10^{-9} and had an overshoot of 4.652% and settling time of 9.134. The gain-scheduled PI controller resulted in lower errors (IAE = 0.26632 and RMSE = 0.03681), less overshoot (3.704%) and shorter settling time (9.078). However, the MFA controller had a slightly higher error value (IAE = 0.27295, RMSE = 0.03756) and a lower overshoot value (1.934%).

The PI controller achieved the least values of IAE and RMSE (0.43612 and 0.05959, respectively) at the highest operating level (1×10^{-8}) with the least overshoot (6.955%) and settling time (9.380). These were reduced to 0.40321 (IAE) and 0.05343 (RMSE) using the gain-scheduled PI controller, along with overshoot of 5.274% and settling time of 9.134. The MFA controller showed IAE of 0.41021 and RMSE of 0.05498, with overshoot of 4.432% and settling time of 9.010 hour.

Conclusions

Key performance indices (IAE, RMSE, overshoot and settling time within a 2% pH tolerance band, 6.86–7.14) were used to compare the performance of the three controllers, fixed PI, gain schedule PI and model-free adaptive (MFA) controllers on pH control in a modelled nonlinear bioprocess.

The performance of the fixed PI controller was found to be the poorest. It had the largest overshoot of approximately 3.09%, the highest tracking error IAE = 0.1147 and RMSE = 0.0313. In addition, it had large settling time of approximately 9.15 hrs after disturbance, which meant that it did not perform well on the nonlinear and time varying process dynamics.

The performance of the gain schedule PI controller was better than the fixed PI controller. It reduced the error to IAE = 0.08724 and RMSE = 0.02328 and lowered the overshoot to 2.29%. The settling time also improved to 8.11 hours. It did show some small transient oscillations, however, and was unable to eliminate the effects of disturbances.

The MFA controller demonstrated the best overall performance. It gave the minimum values of errors with IAE = 0.02597 and RMSE = 0.00604, while also drastically minimising overshoot to 0.50%. The pH of the effluent stayed within the tolerance band during the disturbance, giving the zero-settling time within the criterion defined.

The MFA controller gave more smooth control action with respect to the flow rate of alkali F_{pH} without any abrupt changes like the PI-based controller. This smooth behaviour helped to achieve better stability and damping of oscillations.

The biomass growth profiles were very similar for all controllers and no detrimental impact on the biological process or productivity was observed because of the more effective pH control achieved by the MFA controller.

The controllers' performance was also tested with various levels of gaussian noise. The MFA controller always resulted in the smallest overshoot, significantly lower than the overshoot of the PI and gain-scheduled PI controllers, for example 0.762% when the noise level is 5×10^{-11} and 4.432% when the noise level is 1×10^{-8}. Furthermore, IAE of 0.19460–0.20639 and RMSE of 0.02091–0.02246 were obtained with the smallest tracking errors for lower noise intensities (5×10^{-11} to 1×10^{-9}) at which the MFA controller was tested.

The numerical results demonstrate that the MFA controller is capable of good tracking performance while retaining the system stability under various operating conditions with a significant amount of overshoot reduction. The comparison of the three controllers shows that there are clear differences in the numerical performance of all the metrics evaluated. The gain schedule PI controller is an enhancement of the fixed PI controller that further reduces the tracking error, overshoot and settling time. MFA controller has lower overshoot value and has a better ability to keep the output within the desired tolerance band, and has a smoother control action.

References

1. V. Galvanauskas, "Adaptive pH Control System for Fed-Batch Biochemical Processes," Information Technology and Control, vol. 38, no. 3, pp. 225–231, 2009.
2. G. S. Cheng, "Model-Free Adaptive (MFA) Control," 2005.
3. J. E. Smith, Biotechnology, 4th ed., Cambridge University Press, 2004.
4. A. Nienow, “Reactor engineering in large scale animal cell culture,” Cytotechnology, vol. 50, no. 1–3, pp. 9–33, 2006.
5. M.L Shuler and F. Kargi, Bioprocess Engineering: Basic Concepts, 2nd ed. Prentice Hall, 2002
6. K.G. Carr-Brion, Measurement and Control in Bioprocessing. Elsevier Science Publishers, London, 1991
7. S. D. Cederbaum, G. C. Fareed, M. A. Lovett, and L. J. Shapiro, “Recombinant DNA in medicine,” West. J. Med., vol. 141, no. 2, p. 210, 1984.
8. J. A. Asenjo, Bioreactor System Design, 1st ed. Boca Raton, FL, USA: CRC Press, 1994. doi: 10.1201/9781482277470.
9. S.-Y. Li, R. Srivastava, S. L. Suib, Y. Li, and R. S. Parnas, “Performance of batch, fed-batch, and continuous A–B–E fermentation with pH-control,” Bioresour. Technol., vol. 102, no. 5, pp. 4241–4250, 2011.
10. M. Pčolka and S. Čelikovský, "Algorithms for nonlinear predictive control maximizing penicillin production efficiency," in Proc. 2016 American Control Conference (ACC), Boston, MA, USA, Jul. 2016, pp. 7525460.
11. E. Bolmanis, K. Dubencovs, A. Suleiko, and J. Vanags, “Model predictive control—A stand out among competitors for fed-batch fermentation improvement,” Fermentation, vol. 9, no. 3, p. 206, 2023.
12. P. Oikonomou, "A Probabilistic Batch Oriented Proactive Workflow Management," in Proceedings of the 2021 IEEE 33rd International Conference on Tools with Artificial Intelligence (ICTAI), Washington, DC, USA, 2021.
13. T. Iwasa, N. Morizumi, and S. Omatu, “Temperature control in a batch process by neural networks,” in Proc. 1998 IEEE Int. Joint Conf. Neural Netw. (IJCNN), Anchorage, AK, USA, 1998, pp. 992–995, vol. 2
14. T. Yuan, "Controlling Factors of Shortcut Nitrification in Sequencing Batch Reactor," in Proceedings of the 2010 4th International Conference on Bioinformatics and Biomedical Engineering, Chengdu, China, 2010.
15. M. de Tremblay, M. Perrier, C. Chavarie, and J. Archambault, "Control of Fed-Batch Culture of Hybridoma Cells for Monoclonal Antibody Production," in Proceedings of the 1990 American Control Conference, San Diego, CA, USA, 1990, pp. 2150–2155.
16. M. N. Pantano, M. E. Serrano, M. C. Fernández, S. Rómoli, O. A. Ortiz, and G. J. E. Scaglia, "Tracking multivariable optimal profiles of induced foreign protein production by recombinant bacteria in a fed-batch reactor," in Proceedings of the 2015 XVI Workshop on Information Processing and Control (RPIC), Cordoba, Argentina, 2015, pp. 1–6.
17. Lee and W. F. Ramirez, “Mathematical modeling of induced foreign protein production by recombinant bacteria,” Biotechnol. Bioeng., vol. 39, no. 6, pp. 635–646, 1992.
18. M. Mahmoodi and E. Nassireslami, “Control algorithms and strategies of feeding for fed-batch fermentation of Escherichia coli: a review of 40 years of experience,” Preparative Biochemistry & Biotechnology, vol. 52, no. 7, pp. 823–834, Nov. 2021.
19. B. Partopour and D. Pollard, “Advancing biopharmaceutical manufacturing: economic and sustainability assessment of end-to-end continuous production of monoclonal antibodies,” Trends Biotechnol., vol. 43, no. 2, pp. 462–475, 2025.

20. C. Challener, “Controlling integrated, continuous processes: Real-time monitoring with feed-back and feed-forward controls enables synchronization and enhances robustness,” BioProcess International, Sep. 23, 2024.
21. M. Balat and H. Balat, “Recent trends in global production and utilization of bio-ethanol fuel,” *Appl. Energy*, vol. 86, no. 11, pp. 2273–2282, 2009.
22. D. J. Pollard and J. M. Woodley, “Biocatalysis for pharmaceutical intermediates: the future is now,” *Trends Biotechnol.*, vol. 25, no. 2, pp. 66–73, 2007.
23. R. W. Ellis, “New technologies for making vaccines,” Vaccine, vol. 17, no. 13–14, pp. 1596–1604, 1999.
24. B. Kelley, “Industrialization of mAb production technology: The bioprocessing industry at a crossroads,” *MAbs*, vol. 1, no. 5, pp. 443–452, 2009.
25. D. K. Takma and F. Korel, “Microbial production of aromatic benzaldehydes (cherry and fruit flavors),” in Microbial Production of Food Bioactive Compounds, S. M. Jafari and F. D. Harzevili, Eds. Cham, Switzerland: Springer, 2024. doi: 10.1007/978-3-030-81403-8_54-1.
26. G. Tchobanoglous, H. D. Stensel, and R. Tsuchihashi, *Wastewater Engineering: Treatment and Resource Recovery*, 5th ed., McGraw-Hill, 2014.
27. L. Appels, J. Baeyens, J. Degrève, and R. Dewil, “Principles and potential of the anaerobic digestion of waste-activated sludge,” Prog. Energy Combust. Sci., vol. 34, no. 6, pp. 755–781, Dec. 2008
28. R. C. Saxena, D. K. Adhikari, and H. B. Goyal, “Biomass-based energy fuel through biochemical routes: A review,” *Renew. Sustain. Energy Rev.*, vol. 13, no. 1, pp. 167–178, 2009.
29. T. Tamime and R. K. Robinson, *Yoghurt: Science and Technology*, 3rd ed., CRC Press, 2007.
30. F. Palladino, P. R. F. Marcelino, A. E. Schlogl, Á. H. M. José, R. d. C. L. B. Rodrigues, D. L. Fabrino, I. J. B. Santos, and C. A. Rosa, “Bioreactors: Applications and Innovations for a Sustainable and Healthy Future—A Critical Review,” Appl. Sci., vol. 14, no. 20, p. 9346, 2024
31. C. Kleinstreuer and T. Poweigha, “Modeling and simulation of bioreactor process dynamics,” in Bioprocess Parameter Control, Berlin, Heidelberg: Springer, 2005, pp. 91–146.
32. P. Unrean, "Bioprocess modelling for the design and optimization of lignocellulosic biomass fermentation," Bioresources and Bioprocessing, vol. 3, no. 1, Dec. 2016.
33. Q. Jin, Q. Wu, B. M. Shapiro, and S. E. McKernan, “Limited mechanistic link between the Monod equation and methanogen growth: a perspective from metabolic modeling,” Microbiol. Spectr., vol. 10, no. 2, p. e02259-21, 2022.
34. H. Song, M.-H. Eom, S. Lee, J. Lee, J.-H. Cho, and D. Seung, "Modeling of batch experimental kinetics and application to fed-batch fermentation of Clostridium tyrobutyricum for enhanced butyric acid production," Biochemical Engineering Journal, vol. 54, pp. 1–8, Dec. 2010
35. L. M. Helleckes, J. Hemmerich, W. Wiechert, E. von Lieres, and A. Grünberger, “Machine learning in bioprocess development: from promise to practice,” Trends Biotechnol., vol. 41, no. 6, pp. 817–835, 2023.
36. T. Cui, T. Bertalan, N. Ndahiro, P. Khare, M. Betenbaugh, C. Maranas, and I. G. Kevrekidis, “Data-driven and physics informed modeling of Chinese Hamster Ovary cell bioreactors,” Comput. Chem. Eng., vol. 183, p. 108594, 2024.

37. S. Yang, W. Fahey, and H. Cao, “Hybrid modeling of fed-batch cell culture using physics-informed neural network,” Ind. Eng. Chem. Res., vol. 63, no. 39, pp. 16833–16846, 2024.
38. B. Bayer, M. Duerkop, G. Striedner, and B. Sissolak, “Model transferability and reduced experimental burden in cell culture process development facilitated by hybrid modeling and intensified design of experiments,” Front. Bioeng. Biotechnol., vol. 9, p. 740215, 2021.
39. M. A. Salam, A. Mushtaque, S. Khan, Q.-ul-Ain, A. Younas, N. Fatima, A. Y. Malik, M. A. Shah, and H. Khan, “The effect of glucose, temperature and pH on bioethanol production by Saccharomyces cerevisiae,” J. Popul. Ther. Clin. Pharmacol., vol. 31, no. 1, pp. 656–667, 2024.
40. Uysal, N. Akcan, Z. Baysal, and F. Uyar, “Optimization of α-amylase production by Bacillus subtilis RSKK96: using the Taguchi experimental design approach,” Prep. Biochem. Biotechnol., vol. 41, no. 1, pp. 84–93, 2010.
41. R. Wurm, “Production of recombinant protein therapeutics in cultivated mammalian cells,” Nat. Biotechnol., vol. 22, no. 11, pp. 1393–1398, Nov. 2004.
42. Á. Németh and B. Sevella, “Role of pH-regulation in lactic acid fermentation: Second steps in a process improvement,” Chem. Eng. Process., vol. 50, no. 3, pp. 293–299, 2011
43. A. Kheirolomoom, A. Kazemi-Vaysari, M. Ardjmand, and A. Baradar-Khoshfetrat, “The combined effects of pH and temperature on penicillin G decomposition and its stability modeling,” Process Biochem., vol. 35, no. 1–2, pp. 205–211, 1999.
44. Y. Yang and Q. Wu, "A neural network PID control for PH neutralization process," 2016 35th Chinese Control Conference (CCC), Chengdu, China, 2016, pp. 3480-3483.
45. A. M. Ismael, “Adaptive power control for wireless communication systems,” M.S. thesis, Al-Nahrain Univ., Baghdad, Iraq, Jun. 2017
46. G. A. Pajunen, "Comparation of linear and nonlinear adaptive control of pH-process," 1985 24th IEEE Conference on Decision and Control, Fort Lauderdale, FL, USA, 1985, pp. 850-852.
47. F. Wan, H. Shang, and L. X. Wang, “Adaptive fuzzy control of a pH process,” in Proc. 2006 IEEE Int. Conf. Fuzzy Syst., Vancouver, BC, Canada, Jul. 2006, pp. 2377–2384.
48. S. Saludes and M. J. Fuente, "Fault tolerant fuzzy IMC control in a PH process," 2003 European Control Conference (ECC), Cambridge, UK, 2003, pp. 659-664.
49. B. C. Eeranna, S. Rao, and G. P. Reddy, “Artificial neural network-based modeling and control of bioreactor,” Int. J. Eng. Res. Technol., vol. 3, no. 8, pp. 117–122, 2014.
50. J. Cesmat and J. McAndrew, "Stay on Target: Improving Process Control through Strategic Cascade Feedback Loop Tuning," *Bioprocessing White Paper*, no. 224, Distek, Inc., North Brunswick, NJ, Mar. 2022.
51. J. W. Cangussu, "Integrating Statistical and Feedback Process Control for the Monitoring of the Software Test Process," Dept. of Computer Science, University of Texas at Dallas, Richardson, TX, USA, pp.697-702, 2003.
52. K. Liu, Y. Fan, and J. Chen, “A model free adaptive control method based on self-adjusting PID algorithm in pH neutralization process,” Chin. J. Chem. Eng., vol. 76, pp. 227–236, 2024.
53. C. König, M. Turchetta, J. Lygeros, A. Rupenyan, and A. Krause, “Safe and efficient model-free adaptive control via Bayesian optimization,” in Proc. 2021 IEEE Int. Conf. Robot. Autom. (ICRA), Xi’an, China, May 2021, pp. 9782–9788.
54. BaiLun Biotechnology Co., Ltd., “Fermenter Solutions | BaiLun Biotechnology Co., Ltd,” Bailun Bioreactor. [Online]. Available: https://fermentorchina.com.
55. Optimus Instruments, “SIP Pilot Scale Bioreactor – 50L, 100L and 200L,” Optimus. [Online]. Available: https://optimus.be/product/sip-pilot-scale-bioreactor-50l-100l-and-200l

Appendices

Appendix 1. Gain Schedules PI Controller

```
function [FpH, Ti_h] = AdaptivePI(pH_sp, pH_meas, OUR)
%#codegen
% Adaptive PI controller (time base in hours)

%% Tunable parameters
Kr     = -2.8e6;    % sign should match "base increases pH" loop
gamma1 = 0.50;
gamma2 = 4.50;

Ts_h   = 0.001;     % controller sample time [h] (0.001 h = 3.6 s)

FpH_min = 0.0;
FpH_max = 0.4;

Ti_min = 1e-4;      % h (avoid divide by zero)
Ti_max = 1.0;       % h (avoid too-slow integral)

%% Persistent integrator state
persistent I
if isempty(I)
    I = 0.0;
end

%% Error
e = pH_sp - pH_meas;

%% Adaptive Ti (hours)
Ti_h = gamma1 / (gamma2 + OUR);

% Clamp Ti for numerical safety
if Ti_h < Ti_min, Ti_h = Ti_min; end
if Ti_h > Ti_max, Ti_h = Ti_max; end

%% Candidate (unsaturated) PI output
I_new = I + Ts_h * e;
FpH_unsat = Kr * e + (Kr / Ti_h) * I_new;

%% Saturation + simple anti-windup
FpH = FpH_unsat;
if FpH > FpH_max
    FpH = FpH_max;
elseif FpH < FpH_min
    FpH = FpH_min;
else
    % only accept integrator update when not saturated
    I = I_new;
end
end
```

Appendix 2. Model-Free Adaptive (MFA) Controller

```
function [FpH, e, o] = MFA_Controller(pH_sp, pH_meas)
%#codegen
% MFA + PI-like integral action for pH control (base addition)
%
% Inputs:
%   pH_sp   : pH setpoint (e.g., 7)
%   pH_meas : measured pH (MUST be taken after -log10 block + sensor TF,
%             and ideally through a Unit Delay to avoid algebraic loop)
%
% Outputs:
%   FpH : base (alkali) flow rate [L/h] saturated to [0, 0.4]
%   e   : normalized control error (monitoring)
%   o   : MFA internal output (monitoring)

%% ===== User tuning parameters =====
Ts_h = 0.001;      % sample time [h] (0.001 h = 3.6 s)
N    = 6;          % neurons / memory depth

% Start with these (safe):
Kc   = 0.15;       % proportional gain on (e + o)
Ki   = 0.9;       % integral gain on e
eta  = 0.0;       % learning rate (reduce if oscillatory)

% Actuator limits
FpH_min = 0.0;
FpH_max = 0.4;

% Small bias to avoid "stuck at 0" in base-only systems
FpH_bias = 0.0;   % [L/h] (0.01–0.05 typical)

% Error normalization (keeps e roughly within [-1, 1])
e_scale = 1.0;     % pH units

% Safety clamp on normalized error
e_clamp = 3.0;

%% ===== Persistent states =====
persistent E w h I initialized
if isempty(initialized)
    E = zeros(N,1);     % error memory
    w = zeros(N,N);     % hidden weights
    h = zeros(N,1);     % output weights
    I = 0.0;            % integral state
    initialized = true;
end

%% ===== Error (normalized) =====
e_raw = (pH_sp - pH_meas);           % positive => pH below SP => add base
e = e_raw / max(e_scale, 1e-6);

% Clamp normalized error
if e >  e_clamp, e =  e_clamp; end
if e < -e_clamp, e = -e_clamp; end

%% ===== Integral of error (PI behavior) =====

I = I + Ts_h * (e);
```

```
%% ===== Update error memory vector E =====
for k = N:-1:2
    E(k) = E(k-1);
end
E(1) = e;

%% ===== Hidden layer (logistic sigmoid) =====
p = zeros(N,1);
for j = 1:N
    s = 0.0;
    for i = 1:N
        s = s + w(i,j) * E(i);
    end
    p(j) = s + 1.0;   % bias
end

q = zeros(N,1);
for j = 1:N
    q(j) = 1.0 / (1.0 + exp(-p(j)));
end

%% ===== Output layer =====
o = 0.0;              % bias
for j = 1:N
    o = o + h(j) * q(j);
end

%% ===== Control law (MFA + PI-like integral) =====
FpH_unsat = FpH_bias + Kc*(e+o) + Ki*I;

%% ===== Saturation =====
FpH = FpH_unsat;
if FpH > FpH_max, FpH = FpH_max; end
if FpH < FpH_min, FpH = FpH_min; end

%% ===== Anti-windup for integrator =====
% If saturated and error would push further into saturation, undo the last integration
if (FpH >= FpH_max && e > 0) || (FpH <= FpH_min && e < 0)
    I = I - Ts_h * e;
end

%% ===== Learning with anti-windup (only learn when NOT saturated) =====
if abs(FpH - FpH_unsat) < 1e-12
    eta_eff = eta * Ts_h;

    % sum_h = sum(h)
    sum_h = 0.0;
    for k = 1:N
        sum_h = sum_h + h(k);
    end

    % Update w(i,j)
    for j = 1:N
        dj = (-eta_eff) * Kc * e * q(j) * (1.0 - q(j)) * sum_h;
        for i = 1:N
            w(i,j) = w(i,j) + dj * E(i);
        end
    end

    % Update h(j)
```

```
    for j = 1:N
        h(j) = h(j) + eta_eff * Kc * e * q(j);
    end
end

end
```