# THE pHAUXOSTAT

by

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Submitted in partial fulfilment of the requirements for the degree

of

Doctor of Philosophy

in

The Faculty of Engineering, The Built Environment and Information Technology

University of Pretoria

Pretoria

Study leader : Professor W.A. Pretorius

September 2001

**THE pHAUXOSTAT**

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## ABSTRACT

The pHauxostat technique for process control was proposed in the late nineteen fifties with a theoretical explanation done by Martin and Hempfling in 1976. The theory was extended in 1985 (Rice & Hempfling), but concluded to be incomplete. The objective of this study was to develop a theory for the pHauxostat and to investigate and explain the principles involved. This was done by investigating the pH, as the controlled output variable, and the control methodology with the feed system the manipulated input variable. Laboratory test work was conducted to verify a proposed theory by using a chemically defined substrate. The technique was thereafter applied in treating a petrochemical effluent in a demonstration plant, demonstrating the generality and applicability of the theory and the pHauxostat technique.

The controlled pH of the reactor solution was found to be a function of the weak acids and bases in the reactor solution and the strong acids and bases added to the substrate, in combination with the chemical species removed from the substrate during biodegradation. A method proposed by Loewenthal *et al.* (1991) that was developed for chemical conditioning, utilising solution and subsystem alkalinities, proved to be successful in characterising the reactor solution in combination with traditional equilibrium chemistry.

The pHauxostat control system was shown to keep the alkalinity constant, resulting in a controlled and constant difference in solution alkalinity between the reactor and the substrate solutions. The feed rate is controlled by this difference in combination with the alkalinity generation rate. The alkalinity generation rate is defined with a proposed alkalinity yield coefficient, linking water chemistry and growth kinetics. The alkalinity yield coefficient indicates the amount of alkalinity generated per substrate removed, similarly to the conventional growth yield. The alkalinity yield coefficient was successfully modelled by a theoretical alkalinity yield coefficient, based on oxidation-reduction half reactions as developed by McCarty (1975). This was shown to be true when the change in alkalinity is mainly due to substrate removal.

The developed theory is based on alkalinity, modelling the pHauxostat technique by completing a mass balance on solution alkalinity. The model proved to accurately predict the results for the

laboratory and the demonstration plant test work. The model is represented by the following formula, respectively for layouts of a chemostat and a CSTR with biomass separation:

$$i_s X_{COD}\, Y_{ALK} / Y_{obs} = S_{ALK} - S_{ALK0}$$

and $$i_s X_{COD}\, Y_{ALK}\, (\tau/\theta_c) / Y_{obs} = S_{ALK} - S_{ALK0}$$

The growth limiting nutrient (S) may be a part of a weak acid/base subsystem or not, implicating two methods of control. pHauxostats were categorised on this basis, giving Category A pHauxostats with S = f(pH) and Category B pHauxostats with S ≠ f(pH). The process for Category A pHauxostats is controlled by the concentration of the growth limiting nutrient (determined by the set point pH and the substrate composition), in combination with the difference in the solution alkalinities between the substrate and reactor solutions. The growth limiting nutrient concentration for Category B pHauxostats, is not controlled but is a result of the control system which is determined by the feed rate of the growth limiting nutrient and the difference in the solution alkalinities.

The main contribution of this study is the analysis of the pHauxostat on an alkalinity basis and the subsequent proposed theory with inclusion of an alkalinity yield coefficient. The alkalinity yield coefficient is universal for biological processes in general. Calculation methods for chemical characterisation of the reactor solution were determined together with a method to predict the alkalinity yield coefficient by a theoretical alkalinity yield coefficient. The control methodology was disclosed and pHauxostats were categorised. This study makes the modelling of the pHauxostat technique possible and the implementation thereof, available to the water industry.

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## SAMEVATTING

Die pHauxostat tegniek is in die laat negentien vyftigs voorgestel met 'n teoretiese beskrywing deur Martin en Hempfling in 1976. Die teorie is in 1985 verbeter, maar met die gevolgtrekking dat dit nie volledig is nie. Die doelstelling van hierdie studie was om die teorie te verbeter en die beginsels van die beheermetode te verklaar. Dit is gedoen deur die beheerde uitset-veranderlike, die pH, en die beheermetode van die gemanipuleerde inset-veranderlike, die voertempo, te ondersoek. Laboratoriumtoetse is met ‘n chemies-gedefinieerde substraat voltooi om 'n voorgestelde teorie te verifieer. Die tegniek is ook in 'n demonstrasie-aanleg toegepas, met 'n petrochemieseuitvloeisel as substraat, om die algemeen toepasbaarheid van die teorie en die tegniek te demonstreer.

Dit is gevind dat die beheerde reaktor pH 'n funksie is van die swak sure en basisse in oplossing en die sterk sure en basisse in die substraat, in kombinasie met die chemiese spesies wat uit die substraat verwyder word deur biodegradering. Die reaktoroplossing kon suksesvol gekarakteriseer word met tradisionele ewewigschemie-metodes in kombinasie met 'n metode deur Loewenthal *et al.* (1991) voorgestel (vir chemiese kondisionering), wat gebaseer is op oplossing- en subsisteem-alkaliniteit.

Die beheersisteem hou die alkaliniteit in die reaktoroplossing konstant en gevolglik ook die verskil in die alkaliniteit tussen die reaktor- en substraatoplossings. Die voertempo word beheer deur hierdie verskil in kombinasie met die produksietempo van alkaliniteit. Die produksietempo van alkaliniteit word gedefinieer met 'n alkaliniteits-opbrengs-koëffisiënt, waardeur water chemie en groeikinetika gekoppel word. Die alkaliniteits-opbrengs-koëffisiënt verteenwoordig die alkaliniteit wat gegenereer word per substraat verwyder, soortgelyk aan die konvensionele selopbrengs-koëffisiënt. Die alkaliniteits-opbrengs-koëffisiënt kon suksesvol met 'n teoretiese alkaliniteits-opbrengs-koëffisiënt gemodelleer word, wat op oksidasie-reduksie halfreaksies gebaseer is, voorgestel deur McCarty (1975). Die gebruik daarvan is korrek indien die alkaliniteits-opbrengs hoofsaaklik aan substraat verwydering toegeskryf kan word.

Die voorgestelde teorie word gebaseer op alkaliniteit, waardeur die pHauxostat gemodelleer word deur 'n massabalans op alkaliniteit te voltooi. Die resultate van die laboratoriumtoetse en die

demonstrasieaanleg is suksesvol deur die model voorspel en word deur die volgende formules voorgestel, onderskeidelik vir uitlegte van 'n chemostaat en 'n volledig vermengde mengvat reaktor met selhersirkulasie :

$$i_s X_{COD}\, Y_{ALK} / Y_{obs} = S_{ALK} - S_{ALK0}$$

en $$i_s X_{COD}\, Y_{ALK}\, (\tau/\theta_c) / Y_{obs} = S_{ALK} - S_{ALK0}$$

Die groeibeperkende nutrient (S) kan óf deel uitmaak van 'n swaksuur/basis subsisteem óf nie, wat twee moontlike beheermetodes impliseer. pHauxostate is op grond hiervan geklassifiseer met $S = f(pH)$ vir 'n Kategorie A pHauxostat, en $S \neq f(pH)$ vir 'n Kategorie B pHauxostat. Die voertempo vir 'n Kategorie A pHauxostat word deur die konsentrasie van die groeibeperkende nutrient beheer, en word bepaal deur die beheerde pH-waarde en die substraat samestelling, in kombinasie met die verskil in die alkaliniteit tussen die reaktor- en substraatoplossings. Die groeibeperkende nutrient konsentrasie vir 'n Katergorie B pHauxostat word nie beheer nie maar is die gevolg van die beheersisteem, wat bepaal word deur die voertempo van die groeibeperkende nutrient en die verskil in die alkaliniteit tussen die reaktor- en substraatoplossings.

Die belangrikste bydrae van hierdie studie is die analisering van die pHauxostat op 'n alkaliniteits basis en die gevolglike voorgestelde teorie, met die insluiting van 'n alkaliniteits-opbrengs-koëffisiënt. Die alkaliniteits-opbrengs-koëffisiënt is universeel en kan in modellering van biologiese prosesse in die algemeen gebruik word. Die berekeningsmetodes vir die karakterisering van die reaktoroplossing is bepaal en 'n teoretiese alkaliniteits-opbrengs-koëffisiënt is ontwikkel vir die voorspelling van die alkaliniteits-opbrengs-koëffisiënt. Die beheermetode van die pHauxostat word in die studie verklaar en pHauxostate word gekategoriseer. Hierdie studie maak die modellering van die pHauxostat en die toepassing daarvan moontlik.

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# LIST OF ACRONYMS AND SYMBOLS

## A. ACRONYMS:

| Acronym | Definition | Page no. of first reference |
|---|---|---|
| BOH | Base (type not specified) | 104 |
| COD | Chemical Oxygen Demand | 2 |
| CSTR | Continuous Stirred Tank Reactor | 4 |
| DO | Dissolved Oxygen | 7 |
| GLN | Growth Limiting Nutrient | 7 |
| HA | Acid (type not specified) | 104 |
| HAc | Acetic Acid | 17 |
| HRT | Hydraulic Residence Time | 5 |
| PID | Proportional-Integral-Derivative | 13 |
| RO | Oxygen uptake | 86 |
| SRT | Solids Retention Time | 6 |
| SCFA | Short Chain Fatty Acid | 17 |
| TSS | Total Suspended Solids | 62 |
| VSS | Volatile Suspended Solids | 62 |
| [ANC] | Acid Neutralising Capacity | 22 |

## B. SYMBOLS:

| Symbol | Definition | Unit | Page no. of first reference |
|---|---|---|---|
| b | Decay coefficient | $T^{-1}$ | 5 |
| $C_x$ | Total species x concentration | $M_{mole}\ L^{-3}$ | 18 |
| D | Dilution rate | $T^{-1}$ | 9 |
| $f_D$ | Fraction of active biomass contributing to biomass debris | - | 84 |
| $f_e$ | Fraction of electron donor used for energy | - | 37 |

| Symbol | Definition | Unit | Page no. of first reference |
|---|---|---|---|
| $f_s$ | Fraction of electron donor captured through synthesis | - | 37 |
| $f_x$ | Activity coefficient x | - | 19 |
| F | Volumetric flow rate | $L^3T^{-1}$ | 5 |
| $F_o$ | Influent volumetric flow rate | $L^3T^{-1}$ | 5 |
| $F_w$ | Volumetric flow rate of biomass wastage stream | $L^3T^{-1}$ | 6 |
| $i_s$ | Substrate conversion factor | $M_{mole}M_{COD}^{-1}$ | 35 |
| $K_s$ | Half-saturation coefficient for substrate | $ML^{-3}$ | 3 |
| $K_x$ | Thermodynamic dissociation equilibrium constant x | - | 19 |
| $K'_x$ | Apparent dissociation equilibrium constant x | - | 19 |
| Q | Air flow rate | $L^3T^{-1}$ | 5 |
| $r_{ALK}$ | Reaction rate for alkalinity production | $M_{mole}L^{-3}T^{-1}$ | 35 |
| $r_{so}$ | Reaction rate for dissolved oxygen | $ML^{-3}T^{-1}$ | 97 |
| $r_s$ | Reaction rate for soluble substrate | $ML^{-3}T^{-1}$ | 2 |
| $r_{XB}$ | Reaction rate for active biomass | $ML^{-3}T^{-1}$ | 2 |
| $r_{XD}$ | Reaction rate for biomass decay | $ML^{-3}T^{-1}$ | 114 |
| R | Overall stoichiometric equation | - | 37 |
| $R_a$ | Half-reaction for the electron acceptor | - | 37 |
| $R_c$ | Half-reaction for cell material | - | 37 |
| $R_d$ | Half-reaction for electron donor | - | 37 |
| RO | Mass rate of oxygen utilisation | $MT^{-1}$ | 86 |
| S | The growth limiting nutrient concentration | $ML^{-3}$ | 45 |
| $S_A$ | Acetic acid concentration | $ML^{-3}$ | 27 |
| $S_{ALK}$ | Solution alkalinity reactor | $M_{mole}\ L^{-3}$ | 25 |
| $S_{ALK0}$ | Solution alkalinity feed | $M_{mole}\ L^{-3}$ | 25 |
| $S_o$ | Dissolved oxygen concentration | $ML^{-3}$ | 5 |
| $S_s$ | Soluble substrate concentration | $ML^{-3}$ | 3 |
| $S_{so}$ | Influent soluble substrate concentration | $ML^{-3}$ | 5 |

| Symbol | Definition | Unit | Page no. of first reference |
|---|---|---|---|
| V | Reactor Volume | $L^3$ | 5 |
| X | Biomass concentration | $ML^{-3}$ | 5 |
| $X_B$ | Active biomass concentration | $ML^{-3}$ | 2 |
| $X_{COD}$ | Biomass concentration in COD units | $ML^{-3}$ | 35 |
| x | Alkalinity production for the electron donor and acceptor per unit substrate consumed | $M_{mole}M^{-1}{}_{mole}$ | 39 |
| y | Alkalinity production for cell synthesis minus that of the acceptor per unit substrate consumed | $M_{mole}M^{-1}{}_{mole}$ | 39 |
| Y | True growth yield | $M_{COD}M^{-1}{}_{COD}$ | 2 |
| $Y_{ALK}$ | Alkalinity yield | $M_{mole}M^{-1}{}_{mole}$ | 35 |
| $Y_{TALK}$ | True alkalinity yield | $M_{mole}M^{-1}{}_{mole}$ | 39 |
| $Y_{obs}$ | Observed growth yield | $M_{COD}M^{-1}{}_{COD}$ | 5 |
| $\theta_c$ | Solids retention time | T | 6 |
| $\mu$ | Specific growth rate coefficient | $T^{-1}$ | 2 |
| $\mu_m$ | Maximum specific growth rate coefficient | $T^{-1}$ | 3 |
| $\tau$ | Hydraulic residence time | T | 5 |
| [ ] | Mass concentration | $M_{mole}L^{-3}$ | 18 |
| ( ) | Activity concentration | $M_{mole}L^{-3}$ | 19 |

# DANKBETUIGING

“Aan Hom wat op die troon sit, en aan die Lam, behoort die lof en die eer, die heerlikheid en die krag, tot in alle ewigheid.” Openbaring 5:13

Aan my vrou, Jessie, en my kinders, Liezel, Carla en Simonet, dankie vir die opoffering, ondersteuning en geduld gedurende ‘n belangrike tydperk in julle lewens.

# THE pHAUXOSTAT

## CHAPTER I - INTRODUCTION

### 1. INTRODUCTION

Continuous cultivation in fermentation only started to be successful in the middle of the 20th century (Aiba *et al.* 1965). Hospodka (1966b) ascribed the slow development due to the lack of fundamental knowledge of growth and multiplication of microorganisms. He pointed out that the theory for homogeneous continuous cultivation was only developed in 1950. Probably referring to a publication by Monod in 1950, titled; "La technique de culture continue" (Monod 1950).

The empirical Monod equation describing the relationship between the specific growth rate and the concentration of an essential growth nutrient was published in 1942 (Monod 1942). This laid the basis for modelling continuous culture cultivation. Herbert *et al.* (1956) completed an experimental study explaining the theory of continuous culture with reference to Monod's proposed relationships and formula. They reasoned that the theoretical background needs to be solved before the technique can intelligently be applied, giving the lack of acceptance of the theory as a reason for the neglect of the technique. They used the chemostat (Novick & Szilard 1950) for the explanation of the theory and their experimental studies. Ironically, today the chemostat has become the most widely used apparatus for studying microorganisms under constant environmental conditions (Gottschal 1990).

The pHauxostat emanated from the chemostat and is an innovative culture control technique. It was first proposed by Wilkowske & Fouts (1958) but brought to the forefront by Martin & Hempfling (1976). Since then only a limited number of studies utilising this technique were undertaken. This may be noticed by completing a literature search, generating only a few references. Gottschal (1990) also expressed surprise by the limited number of studies undertaken using the technique. The reason for the limited use, with no known full scale application, is possibly the lack of understanding of the theoretical background, similarly as described above for the chemostat. A second reason might be that the technique is found difficult and impractical, as was thought to be the case for continuous culture (Herbert *et al*. 1956). Should this be the case, then it is also due to a

lack of understanding, as the control technique proves to be very effective and surprisingly easy to use. The technique certainly has potential for a number of applications with improved process control and increased efficiency.

The purpose of this study is to investigate, determine and explain the principles involved in the pHauxostat technique, thereby progressing in the theoretical handling of the topic.

## 2. LITERATURE REVIEW AND BACKGROUND

*To investigate the principles involved in the pHauxostat technique, it is necessary to briefly cover growth kinetics, modelling and control, all of which are applied in the pHauxostat.*

### 2.1 Growth kinetics

Growth may be described through catabolic and anabolic pathways by which cell material is synthesised with an associated electron exchange (Lim 1998). In short, substrate is utilised to derive energy, building blocks (nutrients) and reducing power (for electron exchange) from it, with an ultimate transfer of electrons to a terminal electron acceptor. Biomass is produced from these products. Combined, substrate is utilised or consumed and biomass is produced, with a proportionally factor, the true growth yield (Y), coupling the two overall biochemical reactions. Growth may be expressed as (Grady *et al.* 1999):

$$r_{XB} = -Yr_S \qquad (1)$$

with $\mathbf{r_{XB}}$ **the rate of biomass production** and $\mathbf{r_s}$ **the rate of substrate consumption** with **Y** the **true growth yield**, all expressed in units of chemical oxygen demand (COD). The rate of substrate consumption may be expressed by:

$$r_s = -\mu X_B / Y \qquad (2)$$

with $\mu$ **the specific growth rate coefficient** and $\mathbf{X_B}$ **the active biomass concentration**. Monod (1949) proposed an empirical equation describing the interrelationship between the growth rate and

substrate concentration and can be expressed as:

$$\mu = \mu_m S_s / (K_s + S_s) \qquad (3)$$

with **$\mu_m$ the maximum specific growth rate**, **$S_s$ the substrate concentration** and **$K_s$ the half-saturation coefficient for substrate**, which is the substrate concentration at half maximum specific growth rate. The substrate concentration represents the growth limiting nutrient concentration which can be the carbon source, the electron donor, the electron acceptor, or any other factor needed by the organism for growth (Grady *et al.* 1999). The specific growth rate increases as the growth limiting nutrient increases up to the maximum specific growth rate. The equation is generally accepted in literature as a good description of the relationship. The equation is also acceptable for the growth limiting nutrient to be measured in units of COD (Gaudy & Gaudy 1980). The equation is demonstrated in Fig. 1.1.

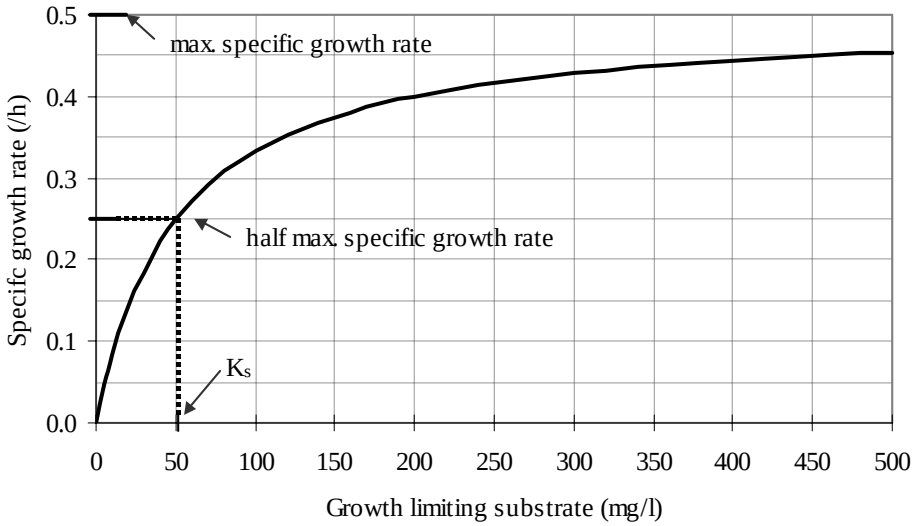


FIG. 1.1 - Graphical presentation of the Monod equation ($\mu_m$ = 0,5 $h^{-1}$, $K_s$ = 50 mg/*l*)

The explanation on growth kinetics is extended in Appendix A.

## 2.2 Bioreactors and Modelling

Bioreactors are generally designed and analysed to be completely mixed reactors (Bailey & Ollis 1986). This has the benefit of uniform conditions and concentrations within the reactor. The

other extreme for reactor design is the plug flow reactor with no mixing. Levenspiel (1999) points out that the completely mixed reactor is more efficient for autocatalytic type reactions, and the plug flow more efficient for reactions decreasing with reaction time. Microorganism growth is an autocatalytic type reaction (Grady & Lim 1980) and hence the general use of completely mixed reactors for bioreactions.

A number of different configurations of completely mixed bioreactors were developed with time, each with its own characteristics (Grady & Lim 1980). Relevant configurations will be discussed.

**Batch Processes**

The batch reactor has probably the simplest configuration and operation. The reactor is filled, seeded and the culture left to grow. Growth proceeds through a number of phases and may be stopped at any stage (Lim 1998). Concentrations vary with time making modelling difficult. Normally no substrate is added once the process has started, making the configuration not suitable for a pHauxostat. An extension of the batch process is the fed batch process. The reactor is only partially filled at start-up whereafter substrate is added to some set programme (Ratledge & Kristiansen 2001). It is a semi-continuous process and may be ideal for a pHauxostat under certain circumstances. These configurations were not investigated in this study and will therefore not be discussed further.

**Continuous Processes**

Probably the best known continuous culture bioreactor is the chemostat (Gottschal 1990). The chemostat was named and described by Novick & Szilard (1950). It is a continuous culture technique utilising a continuous stirred tank reactor (CSTR) and provides a steady state for keeping microorganisms in a well defined physiological condition, ideal for physiological studies.

The chemostat

Shown in Fig. 1.2 is a chemostat or CSTR with an influent and effluent stream and constant volume. Complete mixing is done by mechanical stirrer and/or by gas mixing by the gas supplied for aeration.

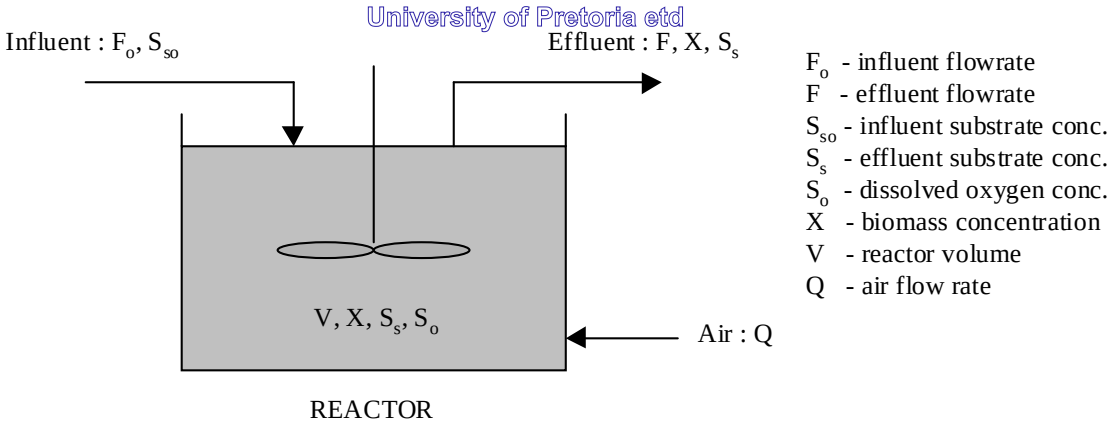


FIG. 1.2 - The chemostat or CSTR

The CSTR and its modelling is well described by Grady & Lim (1980) and may be explained by completing mass balances over the control volume, taken as the reactor volume (V), on; (i) substrate, (ii) biomass and (iii) COD. The mass balances are demonstrated, with equation development, in Appendix A. The chemostat is characterised and may be summarised by the following equations (Appendix A):

$$X_B = Y\ (S_{so} - S_s)\ /\ (1 + b\tau) \quad (4)$$

$$S_s = [K_s\ (1/\tau + b)]\ /\ [\mu_m - (1/\tau + b)] \quad (5)$$

$$\mu = 1/\tau + b \quad (6)$$

with **b** the **decay coefficient** and **τ** the **mean hydraulic residence time** (HRT). The correlation between the true growth yield and the **observed growth yield ($Y_{obs}$)** is given by the following equation, derived from the mass balances:

$$Y_{obs} = Y / (1 + b\tau) \quad (7)$$

The observed growth yield is less than the true growth yield, with the true growth yield defined as yield without any maintenance energy taken into account. $Y_{obs}$ decreases as the maintenance energy gets proportionally bigger (Grady *et al.* 1999).

CSTR with biomass separator

This configuration is a modification from the chemostat. A CSTR with a biomass separator is shown in Fig. 1.3 (Grady *et al.* 1999). The difference, compared to the chemostat, being that two

residence times exists, one for the mean hydraulic residence time ($\tau$) and the second for the biomass residence time or **solids retention time (SRT)** with symbol $\theta_c$.

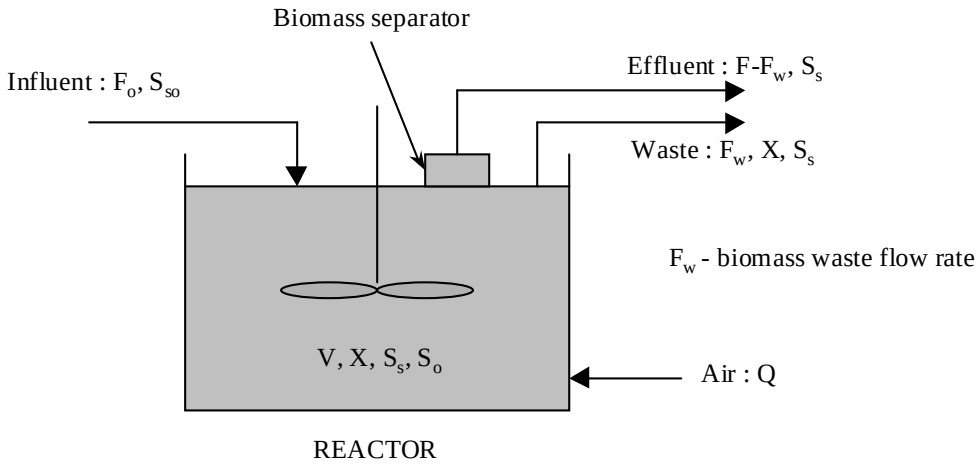


FIG. 1.3 - CSTR with biomass separator

This bioreactor configuration is generally applied in wastewater treatment in which case the biomass separator is normally a settling tank, with recycle back to the CSTR (IAWPRC 1986). The terminology of a CSTR with cell recycle is then used.

This configuration makes it possible to manipulate the SRT while keeping the reactor volume the same, improving process control. On the same basis as for the chemostat the following equations can be derived:

$$X_B = (\theta_c/\tau)\ [Y(S_{so} - S_s)] / (1 + b\theta_c) \quad (8)$$

$$\mu = 1/\theta_c + b \quad (9)$$

$$S_s = [K_s(1/\theta_c + b)] / [\mu - (1/\theta_c + b] \quad (10)$$

## 2.3 Control

Process control started in batch systems with the control of input variables which were not directly related to growth rate control, for example dissolved oxygen concentration, pH, temperature, etc. (Aiba *et al*. 1965). Growth rate in batch systems is normally uncontrolled and at maximum rate at

the start (Ratledge & Kristiansen 2001). Continuous culture cultivation introduced a new dimension, necessitating the control of growth rate. Herbert *et al*. (1956) completed chemostat studies to prove that growth can be controlled at sub-maximum growth rates. This can be done due to growth rate being a function of a growth limiting nutrient (GLN) concentration, as demonstrated by the Monod equation (Eq. 3). There may therefore be a difference in the aim of control, whether the purpose is associated with growth rate control in a given environment, or control of the growth environment, to optimise growth.

**Nomenclature and explanation**

Systems may be described in terms of process variables defined as follows (Olsson & Newell 1999). **Input variables** - classified into **Manipulated** and **Disturbance** variables. Manipulated variables are controlled while Disturbance variables not. **State variables** - independent variables which uniquely determines the state of the process. **Output variables** - variables that can be observed and are related in some way to the State variables. A State variable may also be a Output variable, if it can be observed.

Considering the chemostat in Fig. 1.2 above, the Input variables are $F_o$, $S_{so}$ and Q (the air flow rate) with $S_s$, X and the dissolved oxygen (DO) concentration, the Output variables. Zhao & Skogestad (1997) demonstrated that the available Manipulated Input variables for a chemostat are the variables $F_o$ and $S_{so}$, and the process State variables available for controller design, the Output variables $S_s$ and X (they did not consider aeration). This may be understood by considering Eqs. 3, 4 and 6. With $F_o$ and $S_{so}$ controlled and fixed, it will result in certain $S_s$ and X values for a given bioreactor volume. $F_o$ controls $\mu$ (Eq. 6), resulting in a substrate concentration $S_s$ (Eq.3). The given $S_{so}$ and resulting $S_s$ controls X (Eq. 4).

The chemostat may be operated on an **Open loop** or **Closed loop** operation (Agrawal & Lim 1984). In the Open loop mode; $F_o$ and $S_{so}$ are the Manipulated Input variables which are kept constant at selected values. This will result in self adjusting $S_s$ and X, State and Output variables. In the Closed loop operation; X or $S_s$ is the controlled State variable, controlled to a desired value through manipulation of $F_o$ or $S_{so}$, the Manipulated Input variables, making control more sophisticated.

Control may also be described as **Feed forward** or **Feedback** control. This description relates to the physical position of the measurement point relative to the control point within the flow diagram.

Feed forward being the information flow in the control loop is in the same direction as the process flow through the process (Olsson & Newell 1999). Feed forward control is in many instances the only possible control method for application. An example is the continuous activated sludge process for sewage treatment for which the feed flow rate and feed concentrations are uncontrolled Disturbance variables. For Feedback control, the control information and process information are counter current. Nguyen *et al.* (2000) used a Feedback control in a sequencing batch reactor system for controlling the cycle time in brewery effluent treatment, for example. The DO concentration in the reactor was measured which indicated the end of the batch reaction time with a rapid increase in DO concentration. This was used as signal for the cycle to be ended and fresh feed to be introduced.

The bioreactor configuration for a CSTR with biomass separation, separates the hydraulic retention time and the solids retention time. The substrate concentration in the bioreactor is controlled via the SRT (Eq. 10). The SRT is now a Input variable which is used in wastewater treatment as the Manipulated Input variable. This is not surprisingly, since the other two Input variables, $F_o$ and $S_{so}$, are normally uncontrollable, or Disturbance variables (Olsson & Newell 1999).

As explained above, control may be categorised, defined and described in different ways, but always needs to incorporate the Input variables; $F_o$, $S_{so}$ and $\theta_c$, and the State variables; F, $S_s$ and X in the control methodology for growth rate control.

**Control Configurations**

Many different control configurations were developed with time, each with its own characteristics and ideal application area. These include the conventional chemostat (Novick & Szilard 1950), the Turbidostat (Bryson & Szybalski 1952), the Nutristat (Edwards *et al.* 1972), the pHauxostat (Martin & Hempfling 1976), the Cyclostat (Chisholm *et al.* 1975), control of $CO_2$ concentration (Watson 1969) and control of the oxygen-absorption rate (Hospodka 1966a). Most of these configurations were developed early in the second half of the $20^{th}$ century, as can be noted from the publication dates. The problems experienced with instrumentation and measurement at that stage were reasons for the development of different control configurations (Fuld & Dunn 1957) and the availability of new sensors influenced the time frame of development. Watson (1969) for example developed the control configuration for the measurement of $CO_2$ concentration in the off gas because of the poor reliability of the photoelectric sensor in the Turbidostat configuration. It demonstrates that

successful control depends on successful measurement of the Output variables used. The differences between the different Feedback control configurations, of which the pHauxostat is one, are also essentially differences in the parameter used for measurement and control. Some of these control configurations were analysed for controllability and stabilisability by different authors (Edwards *et al*. 1972; Zhao & Skogestad 1977; Agrawal & Lim 1984; Menawat & Balachander 1991) trying to identify the most effective control configuration and to compare applicability in situations of different disturbances.

Another relevant and important distinction between two types of control configurations needs to be mentioned. The growth rate is directly controlled by the operator in a **External control** configuration. For example controlling the feed rate to a chemostat, thereby controlling the dilution rate that fixes the growth rate, with self-adjusting $S_s$ and X (Herbert *et al*. 1956). It also means that should the growth rate not be fast enough, then the culture will be washed-out. The second configuration is one by which the growth rate is controlled by some kind of internal control (Gottschal 1990) or **Self-regulating control**. This may be done by controlling the GLN concentration, for example by manipulation of $F_o$, resulting in a self-regulated growth rate. Should the growth rate decrease, then the feed rate will decrease and the culture will not be lost.

A few relevant control configurations will be discussed.

The conventional Chemostat

The conventional Chemostat's control configuration has been referred to in examples above and is characterised by a External control with Input variables $F_o$ and $S_{so}$ (Zhao & Skogestad 1997). The value of the chemostat is in its ability to keep the culture in a well-defined physiological condition over long periods of time (Gottschal 1990). An important aspect not mentioned above is the applicable range of operation. Herbert (1959) showed that the range for constant and reliable operation is from a dilution rate (D) of nearly zero ($D \simeq 0{,}03\ h^{-1}$) to a point distinctly below the critical wash-out point (the point at maximum growth rate). The reason is that near wash-out, a small fluctuation in dilution rate or retention time has a major influence on the biomass concentration, as shown in Fig. 1.4. In this region operation becomes erratic.

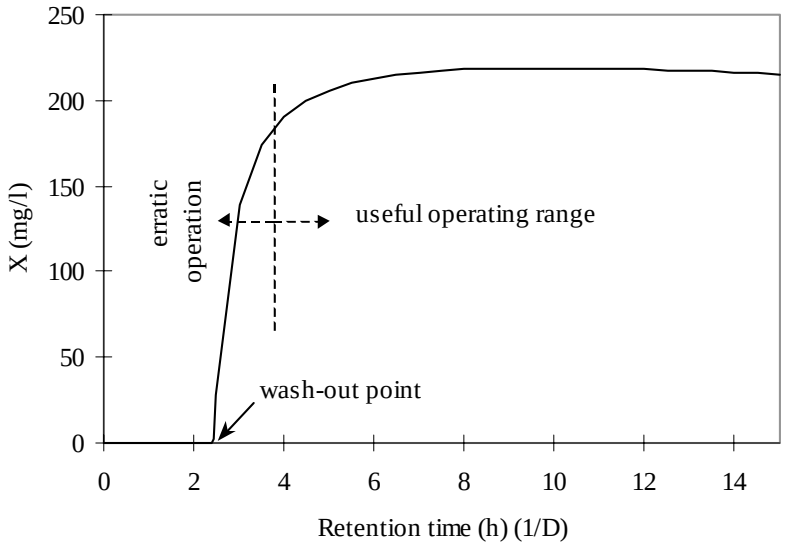


FIG. 1.4 - Steady-state biomass concentration at different retention times for the chemostat

The Turbidostat

Bryson & Szybalski (1952) described the Turbidostat. The Turbidostat is a variation on the Chemostat by which the control configuration was changed. The technique measures the optical density of the bioreactor contents, which relates to the biomass concentration. This signal is used to control the feed rate depending on the set point. The measured and controlled parameter (Output variable) is the optical density (turbidity) which is a measure of the biomass concentration (State variable) and therefore a growth-dependent parameter. This technique controls the State variable, X, by manipulating the Input variable, $F_o$. Manipulating both $F_o$ and $S_{so}$ (Agrawal & Lim 1984) or only $S_{so}$ (Menawat & Balachander 1991) is also possible. It is a Closed loop and Feedback control system and a Self-regulating growth rate type.

The value of this configuration is that the culture cannot be washed-out. The biomass concentration is very sensitive to small changes in growth rate near wash-out (refer Fig. 1.4) and is therefore ideal for operation near maximum growth. The biomass concentration is however relative insensitive to changes in the growth rate at slow growth, making the measured parameter unsuitable in this range.

The Nutristat

Fuld & Dunn (1957) used a control configuration whereby the residual substrate (or GLN) concentration was used as the controlled Output variable. Edwards *et al*. (1972) named it the Nutristat. The control set-up is similar to the Turbidostat with only the controlled Output variable different. The difference is the measurement, which depends on the sensor. Specific sensors have been developed for specific applications, for example an ammonium ion selective electrode was proposed and tested by Suzuki *et al.* (1986) for control in a Nutristat. The success of control will be influenced by the sensitivity of the sensor relative to the required concentration range of the nutrient to be measured. The unavailability of accurate measurement devices hindered the application of the Nutristat (Agrawal & Lim 1984) which seems to be an ideal control configuration.

The Nutristat has the same strong point as the Turbidostat in that operation near wash-out is possible. Control in the slow growth range is however also successful, making it a handy control configuration (Edwards *et al*. 1972). The Nutristat was found to be ideal for physiological studies concerning inhibition and toxicity by Rutgers and co-workers (Rutgers *et al.* 1993; Rutgers *et al.* 1996; Rutgers *et al.* 1998) and by Müller *et al.* (1997).

The pHauxostat

The pHauxostat falls into the same category as the Turbidostat and the Nutristat, but utilise the pH as the controlled Output variable. The pH in the bioreactor is measured and controlled through manipulating the feed flow rate. This control configuration can only work if a change in the pH of the substrate is associated with growth. The pHauxostat is discussed in detail under the next section.

## 2.4 The pHauxostat

The pHauxostat was (as far as could be ascertained) first proposed by Wilkowske & Fouts (1958), for the production of lactic acid in milk fermentation. Girginov (1965) developed something similar to improve the first stage in yoghurt fermentation (Driessen *et al.* 1977). Watson (1972) proposed the terminology “Turbidostat pH” referring to work done at the CSIR, RSA (CSIR 1970a; CSIR 1970b). Martin & Hempfling (1976) were the first to publish a comprehensive study, calling the technique the “phauxostat”. They also completed a mathematical analysis of the process and

proposed a theory. Thereafter only a few more studies using the technique were published, following either the Martin and Hempfling route of calling the technique a phauxostat or just a pH-stat (Stouthamer & Bettenhaussen 1976; Driessen *et al*. 1977; Oltmann *et al.* 1978; Rice & Hempfling 1978 and MacBean *et al.* 1979). Driessen *et al*. (1977) and MacBean *et al*. (1979) also completed mathematical analyses of the technique using a different approach to that of Martin & Hempfling (1976). Rice & Hempfling (1985) improved the Martin and Hempfling theory in 1985. No improvement in the theories was published since then, although Rice & Hempfling (1985) concluded that the theory needs further development.

Concerning terminology, it is general practice to use the terminology "pH-stat" for a system in which the pH in the reactor is kept constant by addition of acid or base. The terminology is used in the chemical engineering field with no relevance to biotechnology by which the substrate feed rate is manipulated by the pH control system. The more appropriate terminology would therefore be "phauxostat" but using a capital H to emphasize and be in-line with the terminology pH. A few authors used this terminology which will also be used in this study as such, therefore "pHauxostat". Martin and Hempfling devised the terminology because it functions by using the pH of the medium to maintain growth (auxo, from the Greek *auxein*, to increase) at a constant (*stat*, from the Greek-*states*, one that causes to stand) (Martin & Hempfling 1976).

The pHauxostat has a control configuration similar to the Turbidostat and Nutristat. It is a Closed loop, Feedback system by which the growth rate is Self-regulated. The Input variables are $F_o$ and $S_{so}$ with $S_s$ and X the State variables and pH the measured Output variable.

**Lay-out**

Different lay-outs are possible but the principle stays the same and can be explained in its simplest form shown in Fig. 1.5. The pH controller controls the feed pump, with pH measurement in the bioreactor and the pH set point at a predetermined value. Changes in the pH occur as a result of substrate conversion or removal, which thereby triggers the feed pump. The addition of substrate corrects the pH and again triggers the pump to stop. Depending on the setting of the pump feed rate, near continuous feed is possible, or by simply using a pH controller with a Proportional-

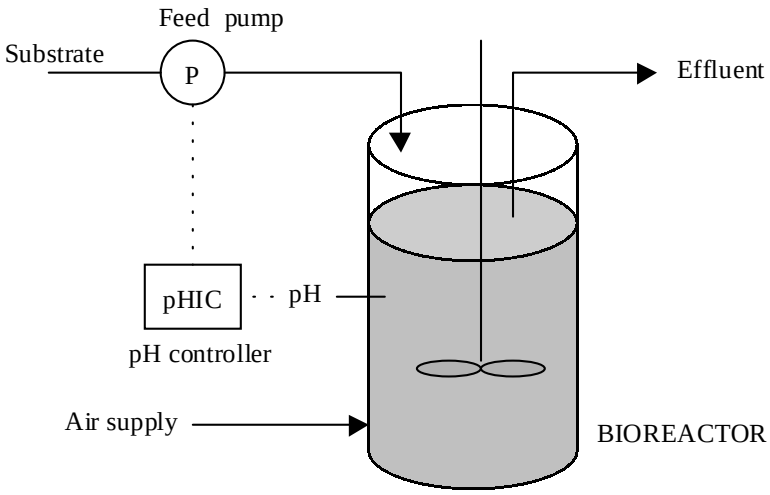


FIG. 1.5 - The pHauxostat lay-out

Integral-Derivative (PID) algorithm control and analog output and an analog input feed pump, a continuous feed rate can be maintained. It is required that the substrate and the bioreactions taking place in the reactor, be such that a pH change results from substrate conversion and that a correlation exists between the microbial growth and the pH change. This results in a Self-regulated feed rate, therefore dilution rate and growth rate, with the pH the controlled growth-dependent parameter.

**Applications**

As mentioned before, the pHauxostat technique was used in fermentation in the dairy industry (Wilkowske & Fouts 1958; Girginov 1965; Driessen *et al*. 1977 and MacBean *et al*. 1979), Martin & Hempfling (1976) and Rice & Hempfling (1985) used it to demonstrate it as an alternative continuous culture technique for physiological studies, which was used as such by Rice & Hempfling (1978), Stouthamer & Bettenhausen (1976) and Sowers *et al.* (1984). Oltmann *et al*. (1978) suggested the technique for continuous mass cultivation of bacteria for the isolation of cellular constituents and was similarly demonstrated as a technique for the production of polyhydroxyalkanoate (Choi & Lee, 1999; Tsuge *et al.* 1999; Sugimoto *et al.* 1999; Kobayashi *et al.* 2000). Kistner *et al.* (1983) used it to determine growth rates of fibrolytic rumen bacteria on particulate medium, which was the only publication found on particulate medium besides it being mentioned in a CSIR report (CSIR 1970b). The technique was also used for improved start-up of high rate anaerobic effluent treatment processes (Brune *et al.* 1982; Fiebig & Dellweg 1985;

Schulze *et al.* 1988; Pretorius 1995; Austermann-Haun *et al.* 1994) and studies on inhibition and toxicity (Demirer & Speece 1999; Demirer & Speece 2000). The application of the technique included both aerobic and anaerobic processes and it is interesting that Martin & Hempfling (1976) demonstrated a smooth transition from aerobic to anaerobic and back to aerobic, using *Escherichia coli*. Most of the studies were continuous cultivation but application also included fed-batch cultivation (Choi & Lee 1999; Tsuge *et al*. 1999; Kobayashi *et al*. 2000).

It is clear from the published studies that the technique has a number of applications and all the authors concluded that the technique worked well with benefits over other techniques. It is labelled as reliable and easy to operate. Martin & Hempfling (1976) brought the technique under world attention in the seventies and not withstanding the good report by different authors, only limited studies were reported thereafter. No full scale or even pilot plant applications were found in the literature. Agrawal & Lim (1984) evaluated different control configurations and mentioned the little attention the technique enjoyed while Gottschal (1990), in a review on continuous culture techniques, expressed surprise in the limited studies done using the technique. The same can be said for the last decade. The reason for this is probably the ill understood theoretical background, as mentioned in the Introduction. The published studies were also done to a certain extent on a black box method, resulting in contradictions by different authors (Rice & Hempfling 1978; MacBean *et al.* 1979).

**Theory development by Martin and Hempfling**

Martin & Hempfling (1976) were the first to propose a theory for the pHauxostat. They considered the change in the proton concentration in the reactor and argued that it must be balanced by the inflow of the substrate. They derived equations from an expression of the rate of change of the proton concentration, with an assumption that the difference in the proton concentrations between the feed and reactor solutions is negligible. For steady state the following equation was derived:

$$xh = BC_R \qquad \text{(Martin \& Hempfling (1976) Eq.5)} \quad (11)$$

with: x - the population density (therefore X)

h - the stoichiometry of proton production related to growth

$BC_R$ - the buffer capacity of the substrate, defined as the amount of acid or alkali required to change the pH of 1 *l* of the substrate to the pH of the reactor

They argued that x should be dependent on $BC_R$ and the growth rate independent thereof. They found that with changing $BC_R$ the growth rate stayed constant and that x was dependent on $BC_R$, but that the value of h changed. Rice & Hempfling (1985) extended the test work showing that under growth limiting conditions the growth rate decreased with an increase in $BC_R$ and not as previously concluded to be independent of $BC_R$ (the previous work was done at maximum growth rate). The value of h also changed unexpectedly over the test range and they concluded that before the theory can be improved, the reason for the variation in the stoichiometry of proton production linked to growth, needs to be understood.

## 2.5 Objective of this study

The objective of this study is to explore and explain the principles involved in the pHauxostat technique and further develop the theory, thereby progressing in the philosophy and the theoretical handling of the topic. This is done by investigating the controlled Output variable, the pH, and the methodology of the feed system.

The technique is first conceptualised, methods for characterisation proposed and a theory developed (Chapter II). The proposals are thereafter verified in laboratory test work in Chapter III and finally demonstrated by treatment of a petrochemical effluent in a demonstration pHauxostat plant (Chapter IV).

# CHAPTER II - THEORY DEVELOPMENT

*In this chapter the pHauxostat technique is explored by applying basic principles in water chemistry and microbiology, by reason, suggesting the principles involved and the control methodology. The process is first viewed as a chemical process (chemo-pHauxostat) and thereafter extended to a biological process (bio-pHauxostat). Based on the findings a theory is proposed, pHauxostats categorised and the associated control methods discussed.*

## 1. CONCEPTUAL PROCESS : THE CHEMO-pHAUXOSTAT

### 1.1 Introduction

To conceptualise the pHauxostat process it is convenient to start with only a chemical reaction, taking place in the reactor. The lay-out in Fig. 1.5 is extended to include an additional pump with manual flow rate control, as shown in Fig. 2.1. The pump is used to add sodium hydroxide to the reactor.

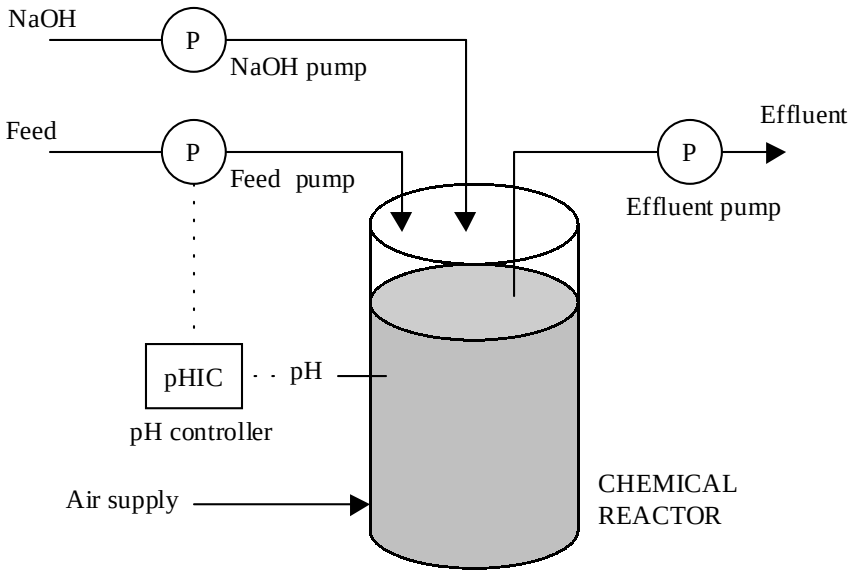


FIG. 2.1 - The chemo-pHauxostat with manual NaOH addition

Assume an acid solution as feed and the addition of NaOH at a fixed rate. As soon as the NaOH is added to the reactor the pH will increase, thereby triggering the feed pump to add feed and decreases the pH. An acid-base neutralisation reaction is taking place in the reactor and the feed-control system regulates it to the preset pH value. **Two aspects are involved in this system that need investigation; the controlled parameter, the pH; and the feed control-methodology**. These two aspects form the basis of the investigation into the principles of the pHauxostat. Both these aspects will be explored and verified with laboratory test work.

## 1.2 The controlled parameter, the pH

The pH of pure water is relative easy to explain and can be calculated from the amount of $H_2O$ molecules in pure water. Explaining and calculating the influence on pH due to different and combinations of solutes become increasingly difficult and complex. Relevant textbooks on the topic include Loewenthal & Marais (1976) on carbonate chemistry, Snoeyink & Jenkins (1980) and Stumm & Morgan (1981) on water and aquatic chemistry.

### Weak acid and base subsystems

pH is influenced by the interactions of acids and bases and the buffer intensity of the solution. The buffer intensity is in turn determined by the weak acid and base subsystems (Snoeyink & Jenkins 1980). In terrestrial waters the carbonate and water weak acids/bases dominate, in municipal wastewater ammonium and phosphate weak acids/bases are present, while in anaerobic treatment systems short chain fatty acid (SCFA) weak acids may dominate (Musvoto *et al.* 1997). This is similar to the solution of some wet-industry wastewater, producing biodegradable organic and acidic effluents with nitrogen and phosphorus added as nutrients. Effluent from a petrochemical industry is an example (Augustyn 1995). Accordingly the weak acids and bases that are important for determining the buffer intensity in these acidic effluents are the water, the carbonate, the phosphate, the ammonium and the SCFA subsystems.

Assume a similar feed to the chemo-pHauxostat with acetic acid (HAc), ammonium chloride and phosphoric acid in distilled water, aerated in the reactor for mixing purposes, similarly to an aerobic system. The pH is determined by equilibrium chemistry of these subsystems (Snoeyink & Jenkins 1980) and will be considered next.

**Equilibrium chemistry of weak acid/base solutions**

Equilibrium chemistry of weak acid/base solutions is associated with the degree of dissociation of the weak acids and bases. Dissociation in turn is dependent on the dissociation constants, the total species concentrations and the ionic strength of electrolyte (Stumm & Morgan 1981). The pH of a solution can be calculated by equilibrium calculations using (i) mass balance equations (total species concentrations), (ii) equilibrium relationships (dissociation constants), (iii) corrected for ionic strength (activity coefficients) and (iv) a proton condition (mass balance on protons) or charge balance (electro neutrality) (Snoeyink & Jenkins 1980). The method for the development of these equilibrium equations is well described in literature and will not be dealt with here. A review and development on the topic were done by Loewenthal *et al.* (1989), Moosbrugger *et al.* (1993a, 1993b and 1993d) and Moosbrugger *et al.* (1993).

The development of the equations for the above mentioned solution is done in Appendix B. The equations are summarised hereunder:

i) Mass balance equations for total species concentrations:

| | |
|---|---|
| $C_{TC} = [H_2CO_3^*] + [HCO_3^-] + [CO_3^{2-}]$ | (Total carbonate species concentration) |
| $C_{TA} = [HAc] + [Ac^-]$ | (Total acetic acid species concentration) |
| $C_{TN} = [NH_4^+] + [NH_3]$ | (Total nitrogen species concentration) |
| $C_{TP} = [H_3PO_4] + [H_2PO_4^-] + [HPO_4^{2-}] + [PO_4^{3-}]$ | (Total phosphorus species concentration) |
| $C_{TNa} = [Na^+]$ (strong base) | (Total sodium concentration) |

where: [ ] molar mass concentration, mol/*l*

$[H_2CO_3^*]$ the sum of dissolved carbon dioxide and carbonic acid = $[CO_2]_{aq} + [H_2CO_3]$ (Stumm & Morgan 1970)

ii & iii) Equilibrium relationships and activity coefficients:

Total species concentrations are determined analytically in a laboratory, giving mass concentration (Standard Methods 1995). To enable equilibrium calculations with mass concentrations the dissociation constants are adjusted with activity coefficients. The hydrogen ion concentration is however determined by a pH measurement, measuring activity, and is an exception and is used without a correction, giving:

$pH = -\log (H^+)$

$(OH^-) = f_m [OH^-]$

| | | | | | |
|---|---|---|---|---|---|
| water species: | $(H^+)[OH^-]$ | = | $K'_w$ | = | $K_w/f_m$ |
| carbonate species: | $(H^+)[HCO_3^-] / [H_2CO_3^*]$ | = | $K'_{C1}$ | = | $K_{C1}/f_m$ |
| | $(H^+)[CO_3^{2-}] / [HCO_3^-]$ | = | $K'_{C2}$ | = | $K_{C2}f_m / f_d$ |
| acetic acid species: | $(H^+)[Ac^-] / [HAc]$ | = | $K'_A$ | = | $K_A/f_m$ |
| nitrogen species: | $(H^+)[NH_3] / [NH_4^+]$ | = | $K'_N$ | = | $K_N/f_m$ |
| phosphorus species: | $(H^+)[H_2PO_4^-] / [H_3PO_4]$ | = | $K'_{P1}$ | = | $K_{P1}/f_m$ |
| | $(H^+)[HPO_4^{2-}] / [H_2PO_4^-]$ | = | $K'_{P2}$ | = | $K_{P2}f_m / f_d$ |
| | $(H^+)[PO_4^{3-}] / [HPO_4^{2-}]$ | = | $K'_{P3}$ | = | $K_{P3}f_d/f_t$ |

where: ( ) activity (active mass) concentration mol/*l*

$f_m$, $f_d$ and $f_t$, monovalent, divalent and trivalent activity coefficients, refer Appendix B

$K_x$ thermodynamic dissociation equilibrium constants, refer Appendix B

$K'_x$ apparent dissociation equilibrium constants, refer Appendix B

$K_w$ thermodynamic ion product constant, refer Appendix B

$K'_w$ apparent ion product constant, refer Appendix B

iv) Proton condition:

The proton mass balance is established with reference to a reference level of protons. The reference level is taken as the species with which the solution was prepared. The species having protons in excess of the reference level are equated with the species having fewer protons than the reference level. This is demonstrated in Appendix B, resulting in the proton balance given below for the considered solution:

$$[Na^+] + [H^+] = [HCO_3^-] + 2[CO_3^{2-}] + [Ac^-] + [NH_3] + [H_2PO_4^-] + 2[HPO_4^{2-}] + 3[PO_4^{3-}] + [OH^-]$$

There are 14 unknown species and 14 equations to solve the solution species concentrations. The total species concentrations $C_{TA}$, $C_{TN}$, $C_{TP}$ and $C_{TNa}$ are known from the preparation of the feed solution or are analytically determined. The total carbonate species, $C_{TC}$, may be determined from

the carbonate alkalinity and a pH measurement (WRC 1986) or as in this case, for an open system, it is a function of the $CO_2$ partial pressure and pH.

Using Henry's law constant, $K_H$, the dissolved $CO_2$ species may be calculated. The ratio of dissolved $CO_2$ to $H_2CO_3$ is fixed and equal to 99,76 : 0,24 at 25°C and is independent of pH and ionic strength (Stumm & Morgan 1970). The $H_2CO_3^*$ concentration may be approximated by the dissolved $CO_2$ concentration, therefore:

$$K_H \rho_{co2} = [CO_2]_{aq} \simeq [H_2CO_3^*]$$

with: $\rho_{co2}$ the partial pressure for $CO_2$

**Characterising the solution**

These equations can now simultaneously be solved for different total species concentrations to yield the concentration of each subsystem chemical species and the pH. The equations were programmed in the spreadsheet program Excel (1998) for MSOffice. The pH was calculated for solutions with different total species concentrations by using the solver function, and compared to measured values of solutions prepared in the laboratory. Appendix B contains the results and spreadsheet printouts of the program. It indicates that the pH, the controlled parameter, is determined by the weak acid and base subsystems and strong acid and/or base added to the solution. The selected pH for the visualised chemo-pHauxostat will thus fix the subsystem species concentrations for a given feed solution composition. It will also be possible to calculate and predict the species concentrations at the selected pH set point by equilibrium chemistry.

**Conclusions**

- A solution composed of weak acid/base subsystems with strong acid or base addition can be characterised by equilibrium chemistry.

- The controlled Output variable, the pH, is a function of the weak acid/base subsystems and added strong acid or base.

- **The pH set point of the pHauxostat will determine the chemical species concentrations of the weak acid/base subsystems for a given feed solution composition.**

## 1.3 Feed control-method

In Fig. 2.1 it is shown that the feed pump is controlled via the pH-controller, controlling the feed rate such that substrate addition balances the apparent rate in pH change and thereby keeping the pH constant. The apparent rate in pH change depends on the rate of chemical species addition that brings the apparent change in pH about (NaOH in this case) plus the chemical reaction rates to establish equilibrium with change in total species concentration.

The chemical reaction rates to establish equilibrium in liquids, especially acid-base reactions, are extremely fast (milliseconds) with gas transfer slower (Stumm & Morgan 1981). The hydration and dehydration reaction of $CO_2$ to attain equilibrium is in the order of seconds. The limiting step to establish equilibrium in the chemo-pHauxostat would be the $CO_2$ transfer between the liquid and gas phase. But even this rate is relative fast compared to the rate of change within the normal operating range of a pHauxostat (chemostat), which is in the order of hours for biological growth rate and HRT. Equilibrium can therefore be assumed to be instantaneous and the apparent rate in pH change directly related to the rate in chemical species addition (NaOH addition).

For steady state operation it will result in the feed pump adding acetic acid at the exact rate to neutralise the addition of the sodium hydroxide and thereby keeping the pH constant. This is similar to a continuous titration taking place, with addition of the same relative amounts determined by a titration test to the set point pH. The system is at steady state concentrations when the pHauxostat is filled to overflow capacity with feed and NaOH added to the set point pH. Any additional NaOH added to the reactor will now result in feed being added to the same amount as for the ratio determined by titration. The titration process is the NaOH being titrated with the feed solution, but is the same and may be viewed as if the feed solution is being titrated with NaOH. Considering a unit time during steady state operation, therefore a unit volume of feed; the pH of this unit volume of feed is increased from the feed pH to the set point pH. **The mass of NaOH necessary to bring this change in pH about depends on the buffer intensity of the feed** (Stumm & Morgan 1981).

**Buffer intensity**

Buffer intensity is the number of moles of strong acid or base required to change the pH of 1 litre of solution by one pH unit (Benefield *et al.* 1982) and is inter related to alkalinity. Alkalinity is a parameter used in water chemistry and is a measure of the proton accepting capacity of a solution measured against the equivalence point of an equivalent solution (WRC 1986). Stumm & Morgan (1981) used the terminology "acid-neutralising capacity", [ANC], and defined it as:

$$[ANC] = \int \beta dpH \qquad \text{(Stumm \& Morgan (1981) chapter 3 Eq. 98)}$$

with $\beta$ the buffer intensity, which is integrated from the solution pH to the equivalence point pH. The difference in alkalinity ([ANC]) between any two pH's is equivalent to the integration of the buffer intensity between those two pH's, and is here defined as the buffer capacity of the solution between those two pH's. This is demonstrated in Fig. 2.2.

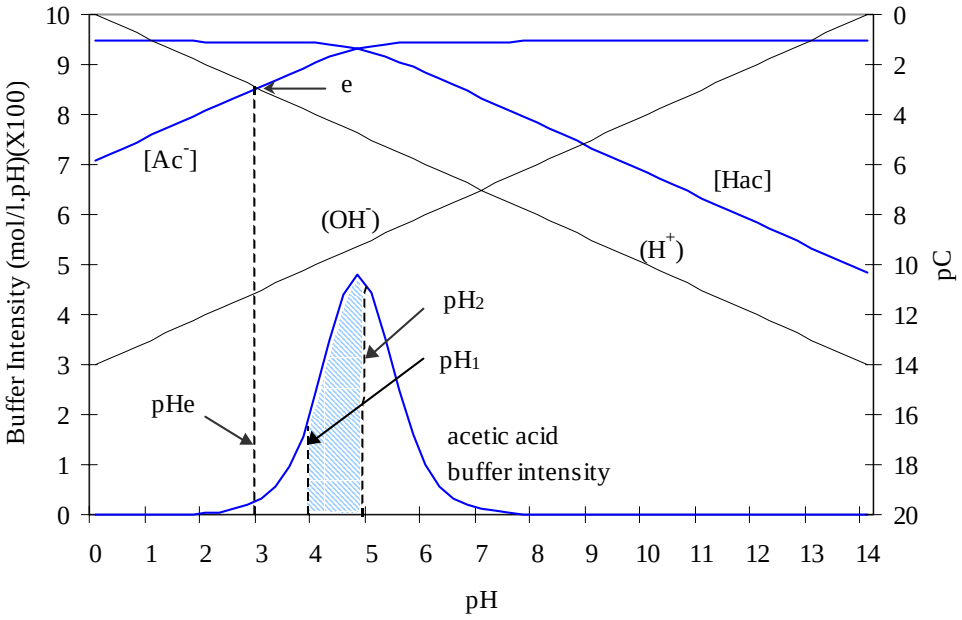


FIG. 2.2 - Alkalinity and buffer capacity

Fig. 2.2 represents a pC-pH diagram combined with the associated buffer intensity for acetic acid and water. The buffer intensity can numerically be expressed by differentiating the equation defining the titration curve with respect to pH (Stumm & Morgan 1981) and is related to the weak acid / base subsystems in solution. Alkalinity is represented by the area under the buffer intensity curve between the solution pH and the equivalence point pH, pHe. The difference between

alkalinity 1 and alkalinity 2, represented by solution $pH_1$ and $pH_2$, respectively, is the area under the buffer intensity curve between $pH_1$ and $pH_2$. This is the buffer capacity of the solution between $pH_1$ and $pH_2$, given by:

$$\Delta\text{alk (alkalinity 2 – alkalinity 1)} = \int \beta d\text{pH} \quad (\text{from } pH_2 \text{ to } pH_1) = \text{buffer capacity}$$

**Defining different alkalinities**

Alkalinity is a measure against the equivalence point of an equivalent solution, as defined above. Different alkalinities can be defined for different equivalent solutions, depending on the reference species, with each alkalinity having its own equivalence point (Loewenthal *et al*. 1989). In terrestrial waters the carbonate subsystem normally dominates which resulted in the general practice to refer to carbonate alkalinity (alkalinity relative to the carbonic acid equivalence point) when using the terminology Alkalinity. In effluents a number of other subsystems may also be present which may include the ammonia, phosphoric and SCFA subsystems, as for the feed solution under discussion. The alkalinity of the feed is a solution alkalinity and is a combination of the different subsystem equivalent solutions, forming one combined equivalent solution with a solution equivalence point. The solution alkalinity is the proton accepting capacity of the solution relative to the solution equivalence point.

Loewenthal *et al.* (1991) defined the solution alkalinity as the sum of the alkalinities of the individual weak acids/bases relative to their respective selected reference species, plus the water subsystem alkalinity. These individual subsystem alkalinities are expressed as "Alk(reference species)" giving the general equation:

$$\text{Solution alkalinity} = \Sigma\, \text{Alk}_i + \text{Alk } H_2O$$

with: $\text{Alk}_i$ - the subsystem alkalinity for the $i^{th}$ weak acid / base subsystem relative to its selected reference species.

Note the difference between HAc alkalinity, and Alk HAc; HAc alkalinity is equal to Alk HAc + Alk $H_2O$. The solution alkalinity for the feed and the reactor solutions can now be defined as:

$$\text{Solution alkalinity} = \text{Alk HAc} + \text{Alk } H_3PO_4 + \text{Alk } NH_4^+ + \text{Alk } H_2CO_3^* + \text{Alk } H_2O$$

with reference species: HAc, $H_3PO_4$, $NH_4^+$, $H_2CO_3^*$ and $H_2O$ respectively,
and:

$$\begin{aligned}
\text{Alk HAc} &= [Ac^-] \\
\text{Alk } H_3PO_4 &= [H_2PO_4^-] + 2[HPO_4^{2-}] + 3[PO_4^{3-}] \\
\text{Alk } NH_4^+ &= [NH_3] \\
\text{Alk } H_2CO_3^* &= [HCO_3^-] + 2[CO_3^{2-}] \\
\text{Alk } H_2O &= [OH^-] - [H^+]
\end{aligned}$$

The SCFA subsystem alkalinities may for simplicity be represented by the acetic acid subsystem alkalinity, because the ionisation constants for the SCFA's typically of concern (acetic, propionic, butyric and valeric) differs only slightly from that of acetic acid (Weast 1974), and the HAc concentration is normally the highest. The SCFA concentrations are converted to HAc concentration and then considered as HAc, giving:

$$\text{Alk SCFA} \underline{\sim} \text{Alk HAc} = [Ac^-]$$

Under Section 1.2 above it was concluded that equilibrium chemistry could be used to characterise the feed and the reactor solutions. All the chemical species concentrations are thereby known and the solution alkalinity can be calculated by using the above equations.

An extended explanation on subsystem alkalinities is given in Appendix C.

**Implication**

In the chemo-pHauxostat the feed addition counteracts the apparent increase in pH above the set point pH by neutralising the effect of the added mass of NaOH. Adding NaOH to the reactor solution also means an increase in the solution alkalinity. The feed addition neutralises or recovers the apparent increase in alkalinity in the reactor, thereby keeping the alkalinity (and the pH) in the reactor constant. The alkalinity of the feed is constant, following that the difference in alkalinity between the feed and the reactor is constant, and kept constant by the control technique. The feed flow rate now depends on the concentration of the alkalinity in the feed and the necessary mass load

of alkalinity to counteract the apparent increase in alkalinity (NaOH addition). Note that the alkalinity in the feed is negative relative to the alkalinity in the reactor. A mass balance on alkalinity over the chemo-pHauxostat can be done, giving:

alkalinity accumulation = alkalinity in - alkalinity out + alkalinity generation.

Although the NaOH is added from an external source it may be viewed as if it is internally generated when the added NaOH liquid volume is negligible compared to the feed volume. Making $NaOH_o$ the NaOH added in units of mol/h and converting it to addition per feed volume (the ratio of NaOH to Feed is fixed for the set point pH), then the alkalinity generation may be defined as:

alkalinity generation per feed volume = $NaOH_o / F_o$

with $F_o$ the feed flow rate in *l*/h.

Completing the mass balance over the chemo-pHauxostat (Fig. 2.1) with V the reactor volume in litre (control volume), $S_{ALK0}$ and $S_{ALK}$ the solution alkalinity in the feed and the reactor, respectively:

$$V\, d\text{alk} / dt = F_o S_{ALK0} - F S_{ALK} + F_o (NaOH_o/F_o)$$
$$\therefore\ d\text{alk} / dt = F_o S_{ALK0} / V - F S_{ALK} / V + (F_o / V)\ (NaOH_o/F_o)$$

For steady state $F_o = F$ and $d\text{alk}/dt = 0$, therefore:

$$NaOH_o/F = S_{ALK} - S_{ALK0}$$
$$\therefore\ F = NaOH_o / (S_{ALK} - S_{ALK0}) \qquad (12)$$

**Eq. 12 indicates that the feed flow rate is determined by the difference in alkalinity between the feed and the reactor (buffer capacity), in combination with the alkalinity generation rate, $NaOH_o$.**

**Conclusions**

- The controlled Output variable for the chemo-pHauxostat is the pH, and the manipulated Input variable the feed flow rate.

- The solution alkalinity is the sum of the subsystem alkalinities relative to its selected reference species and can be calculated using equilibrium chemistry.

- The solution alkalinity in the reactor is kept constant by the control technique and also the difference in solution alkalinities between the feed and the reactor.

- The driving force for the chemo-pHauxostat feed system and the feed flow rate are determined by the difference in the solution alkalinities between the feed and the reactor solutions plus the mass loading rate of base added to the reactor, called the alkalinity generation rate.

# 2. CONCEPTUAL PROCESS : THE BIO-pHAUXOSTAT

## 2.1 Introduction

The chemo-pHauxostat conceptualised in the previous section is actually a normal pH control (pH-stat) for keeping the pH constant in a reactor, except that the normally controlled NaOH addition was exchanged for a controlled feed addition. The terminology "pHauxostat" was incorrectly used as it had no association with biological growth rate control. It was used only to conceptualise the process, with the pHauxostat terminology referring to a bioreactor-pHauxostat.

For a bioreactor-pHauxostat, or bio-pHauxostat, the chemical reaction in the chemo-pHauxostat reactor is supplemented or replaced by bioreactions. The base addition will normally be a natural part of the substrate and/or added to the substrate as part of the feed solution (Martin & Hempfling 1976). The base added to the feed will change the feed alkalinity, and the pH change in the reactor will be brought about by bioreactions. The NaOH pump in Fig. 2.1 falls away giving a lay-out similar to Fig. 1.5.

## 2.2 Conceptualising the bio-pHauxostat

Assume the same feed solution as described for the chemo-pHauxostat as substrate to the bio-pHauxostat, therefore; acetic acid, ammonium chloride, phosphoric acid and sodium hydroxide added to distilled water and aerated in a bioreactor with an added culture. The HAc is assumed the carbon and energy source and the growth limiting nutrient (GLN), with nitrogen and phosphorus macronutrients. Micronutrients are added to the substrate with no or negligible influence on equilibrium chemistry and pH.

The HAc, ammonia, phosphate, carbonate and water species forms the weak acid/base subsystems and together with the sodium hydroxide determines the pH through equilibrium chemistry. The carbonate subsystem for an aerated and acidic solution has a negligible influence on the pH (Loewenthal & Marais 1976). The HAc will decrease the pH with increase in concentration and *vice versa* with all other concentrations constant (Appendix B). It follows that for low concentrations of nitrogen and phosphorus the pH will mainly be determined by the HAc and the NaOH concentrations, with HAc concentration ($S_A$) increasing with an increase in the NaOH concentration at a fixed pH. The pHauxostat control technique controls and keeps the pH constant

implying that it also controls and keeps the $S_A$ constant. The NaOH concentration and the selected pH set point now determine the $S_A$.

**Control of the GLN**

The Monod equation, Eq. 3, demonstrates that the growth rate is influenced by the growth kinetic parameters ($\mu_m$ and $K_s$), but is mainly determined by the growth limiting nutrient concentration. With HAc the GLN it follows that the growth rate is controlled and can be manipulated by the amount of NaOH added and the selected pH set point. The selection of the pH set point is normally determined by process and growth considerations and fixed for optimum process efficiency (Ratledge & Kristiansen 2001). This leaves only the amounts of base added whereby the $S_A$ may be changed and the growth rate manipulated. **It implicates that the growth rate for the bio-pHauxostat is controlled via the pH and may be manipulated by the addition of base to the substrate**. If it is possible to calculate or predict the species concentrations in the bioreactor at the selected pH value, then it will be possible to calculate the $S_A$ for the bio-pHauxostat process and predict the growth rate (with the growth kinetics known).

**The change in pH and buffer capacity**

The apparent increase in the alkalinity and pH by the addition of NaOH in the chemo-pHauxostat is replaced by the bioreaction utilising HAc as substrate in the bio-pHauxostat. The consumption of HAc species will increase the pH to the set point pH where after the control technique will keep it constant. The increase in the pH with consumption of HAc species and the associated decrease in the $S_A$ may in principle be demonstrated graphically by the pC-pH diagram in Fig. 2.3. $pH_1$ represents the equilibrium pH for a HAc concentration $S_{A1}$ with NaOH addition of $[Na^+]$ mol/*l*. The proton condition determining the point of intersection is $[Na^+] + [H^+] = [Ac^-] + [OH^-]$ and may be approximated and simplified to: $[Na^+] = [Ac^-]$ (Snoeyink & Jenkins 1980). Decreasing the HAc concentration to $S_{A2}$ by removal of HAc species will increase the equilibrium pH to $pH_2$, as demonstrated in Fig. 2.3.

Comparing the bio-pHauxostat with the chemo-pHauxostat, a difference exists in that for the chemo-pHauxostat the pH change was without a change in the equivalent solution or equivalence point (no subsystem total species change). The pH change in the bio-pHauxostat is, on the other hand, due to a change in the equivalent solution and removal of weak acid/base subsystem species.

The buffer intensity is represented by the sum of the buffer intensities of all the weak acid/base subsystems in solution (WRC 1992). Changes in the weak acid/base subsystem species will change the buffer intensity. The equivalence point pH and the buffer intensity of the feed solution are changed in the bio-pHauxostat process, with an important implication.

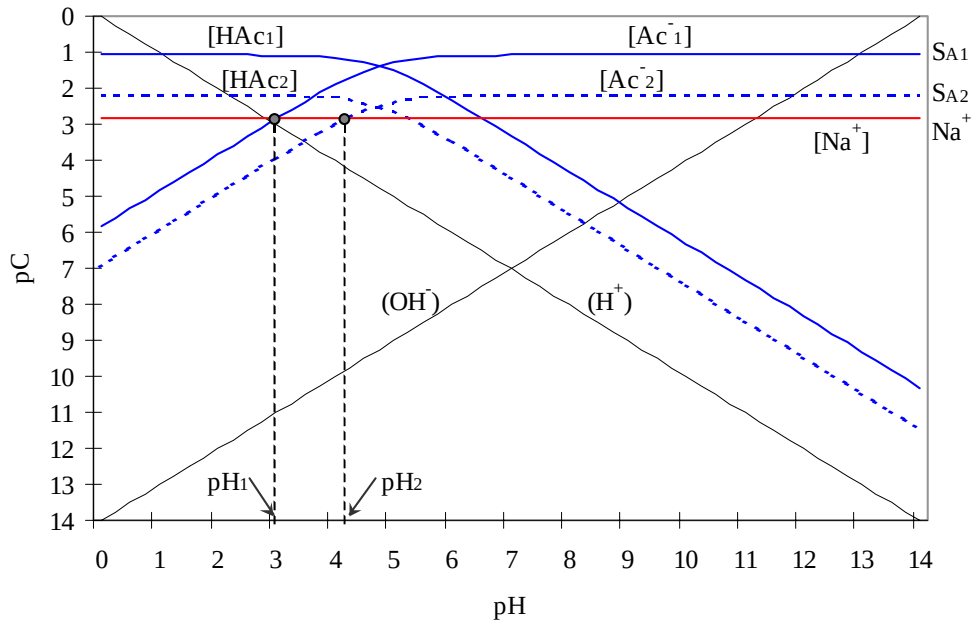


FIG 2.3 - The influence on pH by decrease in HAc concentration

The change in the buffer intensity has the implication that where **the neutralisation effect of the added NaOH in the case of the chemo-pHauxostat was determined by the buffer capacity of the feed solution, it will now be determined by the buffer capacity of the reactor solution**. This will influence the $S_A$ in the reactor and the difference in the pH between the substrate and reactor solutions.

**Aspects to investigate**

The aspects that need clarification to understand the principles involved, are: **the equilibrium chemistry of the bioprocess, the change of alkalinity and pH by bioreactions, and the feed control-methodology**. To describe the pHauxostat mathematically, it will be necessary to incorporate these aspects in a model.

## 2.3 Equilibrium chemistry of the bio-process

In Section 1 above it was shown that equilibrium calculations are done by using mass balance equations, equilibrium relationships and a proton balance. The question is whether this will still hold true for the bio-pHauxostat.

The proton balance is done relative to the input or reference species forming the equivalent solution (Appendix B). The reference species are known for the substrate in this case and a proton balance may be completed. In the reactor the bioreactions remove subsystem species which may and probably are different from the original reference species. A new proton balance is required for the new reference species (or output species) while also taking history into account to keep track of protons. This makes the use of the method impractical, if not impossible for general application and for the bio-pHauxostat.

Loewenthal *et al*. (1991) developed a method by which dosing calculations for the aqueous phase, called chemical conditioning, can be done. It is based on calculations using solution and subsystem alkalinities as defined previously. Relationships between the pH, the subsystem alkalinities and the total species concentrations are developed. Calculation is then possible by the capacity parameters (alkalinity and total species concentration) which change differently with dosing for the subsystem alkalinities and the solution alkalinities. Different calculation sequences are followed depending on the known, the unknowns and the required constituents, between two solutions. The change of the capacity parameters with dosing are fundamental to the sequence of calculation and given by Loewenthal *et al*. (1991) as:

Changes for the subsystem parameters:

- subsystem alkalinities change in a complex fashion with dosing;
- total species concentrations for all subsystems except that including the dosing type remain constant with dosing;
- total species concentration for the subsystem including the dosing type increases by the amount of dosage chemical added.

Changes for solution parameters:

- solution alkalinities that include the dosage type as a reference species do not change with dosing;

- solution alkalinities that do not include the dosage type as a reference species change in a simple stoichiometric fashion with dosing (refer Loewenthal *et al*. (1991)).

Although the method was developed and tested for dosage calculation it is assumed to also hold true for subsystem species removal. **This method is assumed in calculating the subsystem species concentrations for the bio-pHauxostat and to fully characterise the reactor solution**. It implies that with the growth kinetics known the growth rate can be predicted by calculation.

## 2.4 Change in alkalinity and pH by bioreactions

Alkalinity and pH are not changed by bioreactions *per se* but by the associated change in the chemical species. Bioreactions are aimed at deriving energy, building blocks and reducing power from the substrate with an ultimate transfer of electrons to a terminal electron acceptor (Grady *et al*. 1999). Bioreactions are rather categorised by associated nutrient or energy flow and as part of a biochemical cycle or pathway, than focussed on the chemical solution with which it interacts. The solution, with which it interacts, the substrate, determines or not whether alkalinity is available and whether it will be generated or consumed during processing. Moosbrugger *et al.* (1993c) for example divided substrates into two categories, substrates that generate internal buffer, and substrates that do not generate internal buffer. This underlines the fact that **the pHauxostat is similarly only applicable to certain substrates, substrates of which the pH change during biological processing**. Application may be for any bioreaction that brings this change about.

The change in alkalinity during bioprocessing is an important process parameter because of the potential influence on the pH of the process. Notwithstanding this, only a limited amount of information is available on this topic and is rather handled by the necessity of pH control or not. Sam-Soen *et al.* (1991) recommended for example the addition of 1,2 mg $CaCO_3$ alkalinity per mg influent COD, for anaerobic fermentation of carbohydrates, while Ross & Louw (1987) recommended addition of alkali for anaerobic systems with a natural alkalinity below 1000 mg/l as $CaCO_3$. This is not strange considering that modelling of bioprocesses is rather done on an empirical base with unstructured models and is still in an infant stage of development (Blanch & Clark 1997). Models normally do not include the chemical changes in the associated solutions and its influence on pH or alkalinity (IWA 2000).

**Considerations in anaerobic treatment**

The development work done on alkalinity changes during bioprocessing is probably mainly in the field of anaerobic treatment of industrial effluents. The development of high rate anaerobic treatment processes, for example the Upflow Anaerobic Sludge Blanket process, sparked attention and research in this field. Speece (1996), dealing with anaerobic biotechnology, refers to metabolism-generated alkalinity and defines it as the increase of alkalinity resulting from the metabolism of an organic compound with the release of a cation. An example would be a protein, producing $NH_3$ + $CO_2$ and resulting in $NH_4^+$ plus bicarbonate. Carbohydrates, organic acids, aldehydes, ketones and esters are mentioned not to generate alkalinity.

The main consideration in anaerobic processing concerning alkalinity is the availability and production thereof to buffer increased $CO_2$ concentrations (Pretorius 1995) and for buffer against temporal increases in SCFA concentrations (Moosbrugger *et al.* 1993d). Considering the pHauxostat, alkalinity generation is rather viewed as having the effect of an increase in the substrate pH. In this sense organic acids are viewed to generate alkalinity by its biodegradation to carbon dioxide or methane, and water. This may also be defined as acidity removal, but as acidity is the negative to alkalinity, it implies an increase in alkalinity. Note that the focus moved from the reactor solution for the anaerobic process, to the substrate solution for the pHauxostat. Anaerobic fermentation of soluble substrates normally follows two steps, acid formation and then methane production (McCarty & Mosey 1991). The process and substrate is viewed in terms of its end products and not the interim products. In the case of the pHauxostat, carbohydrates may for example be degraded to organic acids in a controlled environment (MacBean *et al.* 1979). This will have the effect of a pH decrease and is viewed as negative alkalinity generation (acidity generation) but was defined by Speece (1996) not to generate alkalinity. Both these cases demonstrate a difference in interpretation of alkalinity generation and highlights careful consideration of the topic.

**Alkalinity change dependent on reference species**

It can be shown that the alkalinity and the associated pH change is related to the reference species used in defining the alkalinity. The HAc alkalinity for a HAc solution with added NaOH will for example stay constant while the pH increases with removal of HAc species. A solution with only HAc species added will have a HAc alkalinity of zero, with a pH at the equivalence point pH. Adding NaOH to the solution means an increase of an equivalent amount of HAc alkalinity.

Removing HAc species from this solution does not change the amount of NaOH added and therefore also not the alkalinity. The alkalinity stays the same but the pH increases as the HAc concentration decreases, demonstrated in Fig. 2.3. Should the alkalinity now be defined with acetate as reference species, measured against the acetate equivalence point, then the removal of HAc species will influence the acetate alkalinity and increase it as the pH increases. This may be demonstrated by including the buffer intensity curve for the solution in Fig. 2.3, shown in Fig. 2.4 below.

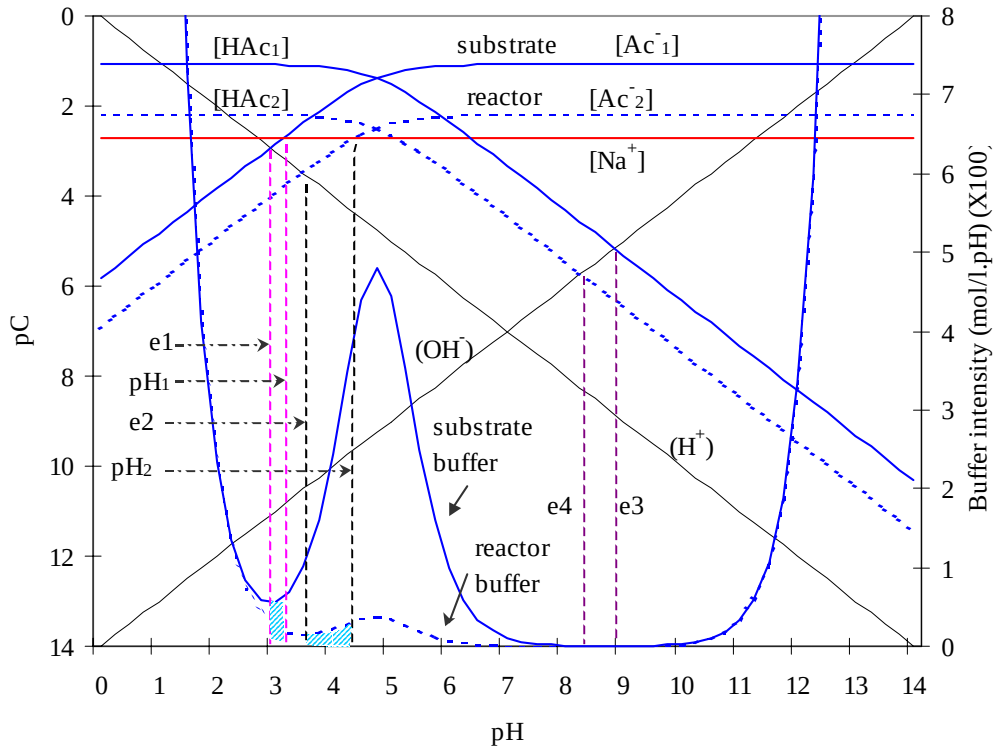


FIG. 2.4 - Change in alkalinity and pH

The buffer intensity curves for both, the substrate solution and the reactor solution (solution with species removed) are shown. The HAc alkalinity is represented by the area under the curves between the pH points, $e_1$ and $pH_1$ for the substrate solution, and $e_2$ and $pH_2$ for the reactor solution. The "e" points refer to the equivalence points. The areas under the curves will be the same. The acetate alkalinity is represented by the area under the curves between the pH points $e_3$ and $pH_1$ for the substrate solution, and $e_4$ and $pH_2$ for the reactor solution. These two areas differ considerably. It demonstrates that **the solution alkalinity does not change with change in reference species but change with change in non-reference species** (Loewenthal *et al*. 1991).

**Conclusion**

The solution alkalinity and pH are influenced by bioreactions due to changes in subsystem species concentrations which influences the equilibrium chemistry, and thereby the pH and alkalinity. The change in alkalinity depends on the reference species used to define the alkalinity. When using alkalinity in calculations as the changing parameter, then the alkalinity defined by species other than the changing species should be used. Together with the previous section it can be shown that the pH and the solution alkalinity is interrelated, and that alkalinity is a parameter that takes the complex influences on pH and its changes into account. pH changes can be modelled by alkalinity and alkalinity can be calculated, as shown in the previous sections.

## 2.5 Theory development

Rice & Hempfling (1985) concluded that before the theory could be improved the reasons for the variation in the stoichiometry of proton production linked to growth, needs to be understood. From the above explanation it is clear that what it implies is that the water chemistry needs to be understood. It also indicates a shortcoming in the developed theory. Another shortcoming is the assumption that the difference in the proton concentration (pH) between the substrate and the reactor solution is negligible. It is precisely this that makes the technique work and should not be ignored, although it may be small in certain circumstances. Maybe the biggest shortcoming is the parameter $BC_R$ or their defined Buffer capacity. By definition it is assumed that the Buffer capacity of the substrate does not change in the process, similar to the chemo-pHauxostat. For the bio-pHauxostat it was however concluded that the important buffer capacity is that of the reactor solution which is different from that of the substrate solution. The Martin and Hempfling theory is probably limited to a few special cases.

To model the pHauxostat it will be necessary to link the feed system and the bioreactions. It was concluded that the feed system can be characterised by the change and difference in alkalinity between the substrate and reactor solutions. A model would therefore need to include alkalinity with linkage to growth.

**Theory based on alkalinity**

The principle followed by Martin & Hempfling (1976) in doing a mass balance on the proton concentration is also followed hereunder, except that alkalinity is used as a parameter. A mass balance on alkalinity over the pHauxostat gives :

$$V.d\text{alk} / dt = F_o.S_{ALK0} - F.S_{ALK} + \text{alkalinity generation rate}$$

$$\therefore \quad d\text{alk} / dt = F_o.S_{ALK0} / V - F.S_{ALK} / V + \text{alkalinity generation rate per volume}$$

$$= F_o.S_{ALK0} / V - F.S_{ALK} / V + r_{ALK} \qquad (13)$$

with $\mathbf{r_{ALK}}$ defined as the **rate of alkalinity generation** in mol/*l*h.

**A new proportionally factor : the alkalinity yield**

Similar to biomass production in Eq. 1, **alkalinity production or generation is defined with a proposed proportionally factor, the alkalinity yield,** $\mathbf{Y_{ALK}}$ , coupling alkalinity generation with substrate consumption, giving:

$$r_{ALK} = i_s \, Y_{ALK} \, r_s \qquad (14)$$

with $\mathbf{Y_{ALK}}$ **the alkalinity yield** in moles alkalinity per moles substrate consumed and $\mathbf{i_s}$ **the substrate conversion factor** of moles per g COD of substrate. $r_s$ is given by Eq. A7[1] and in combination with Eq. A13[1] reveals:

$$r_s = - (F/V) (X_{COD} / Y_{obs})$$

with $X_{COD}$ the biomass concentration in COD units. Substituting $r_s$ in Eq. 14 gives an equation for the rate of alkalinity generation, which is positive, as alkalinity is produced per substrate removed:

$$r_{ALK} = (F/V) (i_s \, X_{COD} \, Y_{ALK} / Y_{obs}) \qquad (15)$$

[1] *Vide* Appendix A p115 & 116

substituting in Eq. 13:

$$d\text{alk} / dt = (F_o/V)\, S_{ALK0} - (F/V)\, S_{ALK} + (F/V)(i_s\, X_{COD}\, Y_{ALK} / Y_{obs})$$

for steady state $d\text{alk} / dt = 0$ and $F_o = F$, giving:

$$i_s\, X_{COD}\, Y_{ALK} / Y_{obs} = S_{ALK} - S_{ALK0} \qquad (16)$$

substituting $Y_{obs}$ with Eq. 7 gives an equation defined with Y:

$$i_s\, X_{COD}\, Y_{ALK}\, (1 + b\tau) / Y = S_{ALK} - S_{ALK0} \qquad (17)$$

Comparing Eq. 16 with Eq. 11[1] from Martin & Hempfling (1976), the similarities are clear. The defined Buffer capacity, $BC_R$, is replaced by the difference in alkalinity between the substrate and reactor solutions but without the limiting assumptions. It incorporates $BC_R$ and the difference in the proton concentration between the feed and reactor solutions, which was assumed to be negligible. The stoichiometric relationship, h, is replaced by $Y_{ALK} / Y_{obs}$ from which it is clear that it will only be constant should the two yield coefficients change proportionally.

The difference in the alkalinity represented by the right hand side of Eqs. 16 and 17 only relates to water chemistry and may be calculated by the developed alkalinity equations and methods described above. Alkalinity yield relates to the substrate consumed, which is associated with biological growth. The substrate consumed is used as the interface between the biological growth and the water chemistry. The question is; what is the value of this proposed proportionally factor, the alkalinity yield, does it change and how, and how is it determined?

## 2.6 Alkalinity yield

The ideal would be to have a similar general formula relating growth to alkalinity, as the Monod equation. Alkalinity, however, relates only to water chemistry with no direct correlation with growth. It is thus not possible.

[1] *Vide* p14

Alkalinity yield may be used, similarly as $Y_{obs}$ is used, to convert and manipulate the units between the substrate consumed and the alkalinity produced. It may be defined per COD of substrate consumed, or per moles/mass of a specific substrate consumed, making it more specific. Alkalinity was previously said to relate to a specific substrate, implying that generalisation is not possible. Alkalinity also has no linkage to COD, making the general and useful simplification and generalisation by the use of COD not possible. Alkalinity yield seems not to be an easy and general definable parameter and not an ideal parameter to use.

**Alkalinity yield and half reactions : a theoretical alkalinity yield**

One possible method besides laboratory test work to determine the correlation between the alkalinity yield and the substrate consumed is the use of half reactions. McCarty (1975) developed a method by which half reactions are used to obtain the stoichiometry of biological reactions. He divided bacterially mediated reactions into a synthesis and an energy component. By considering oxidation-reduction reactions, half reactions are written for electron donors and acceptors for both the synthesis and the energy components, resulting in three oxidation-reduction half reactions. It is done on an electron equivalent basis with the three half reactions; cell synthesis ($R_c$), electron donor ($R_d$) and electron acceptor ($R_a$). The overall reaction, R, is given by:

$$R = R_d - f_e R_a - f_s R_c \qquad (18)$$

with $f_e$ and $f_s$ the fractions of the electron donor coupled with the electron acceptor and cell synthesis, respectively.

The electron donor for the bio-pHauxostat is HAc with oxygen the electron acceptor. The empirical equation for cell material is taken as $C_5H_7O_2N$, which is a generally excepted composition (IAWPRC 1986), but may change depending on the substrate and the growth limiting nutrient (Blanch & Clark 1997). The equation does not contain phosphorus for the reason of simplicity, which only equals approximately one fifth of the nitrogen amount (Grady & Lim 1980), and would unnecessarily complicate the procedure. With ammonia the nitrogen source, the following half reactions describe substrate consumption and cell production for the bio-pHauxostat:

$$R_c: \quad {}^1/_{20}\, C_5H_7O_2N + {}^9/_{20}\, H_2O = {}^1/_5\, CO_2 + {}^1/_{20}\, HCO_3^- + {}^1/_{20}\, NH_4^+ + H^+ + e^-$$

$$R_d: \quad {}^1/_8\, CH_3COO^- + {}^3/_8\, H_2O = {}^1/_8\, CO_2 + {}^1/_8\, HCO_3^- + H^+ + e^-$$

$R_a$: $\frac{1}{2} H_2O = \frac{1}{4} O_2 + H^+ + e^-$

From these reactions it can be seen that alkalinity is produced by the organic donor reaction $R_d$ ($^1/_8$ $HCO_3^-$), but consumed by cell synthesis $R_c$ ($^1/_{20}$ $HCO_3^-$). To calculate the overall reaction it is necessary to determine $f_s$.

The half reactions relate to electron equivalents and so does COD. The fraction, $f_s$, the fraction of the electron donor coupled with cell synthesis, can be expressed as the portion of the feed COD converted to cell mass COD (Grady *et al.* 1999). This is equivalent to the observed growth yield on a COD basis. The moles of alkalinity produced per mole acetate removed or the **theoretical alkalinity** yield is:

$$\begin{aligned} Y_{ALK} &= 8 * {}^1/_8 - 8 * f_s * {}^1/_{20} \\ &= 1 - f_s \,.\, {}^8/_{20} \\ &= 1 - Y_{obs} \,.\, {}^8/_{20} \end{aligned} \qquad (19)$$

**The generality of alkalinity yield**

The yield coefficient defines the change in the solution alkalinity per unit substrate removed and is dependent on four aspects. The one is defined in Eq. 19, indicating its dependence on the observed growth yield, $Y_{obs}$. This is because the observed growth yield is equivalent to $f_s$, as described above, and gives the ratio of the electron flow to either the biomass formed or the electron acceptor (oxygen) and is required in the determination of the overall alkalinity yield. The second dependence is on the substrate or electron donor. Up to this point the same substrate composition was considered, implying that the change in the yield coefficient was only dependent on the growth yield. Should the substrate be different then from the half reactions it is clear that the change in the alkalinity may also be different (refer the electron donor)(McCarty 1975). This will change the yield coefficient. The third dependence is on the electron acceptor and the fourth, the cell synthesis reaction. As for the electron donor the alkalinity change will be different for different acceptors and cell synthesis reactions.

It implicates a specific yield coefficient for each substrate in combination with a bioprocess, similar as for the growth yield coefficient (Grady & Lim 1980). This makes the use of the alkalinity yield coefficient cumbersome.

McCarty (1975) developed a number of half reactions for electron donors, electron acceptors and for cell synthesis reactions. Only two reactions are defined for cell synthesis, depending on the nitrogen source. The electron acceptor is dependent on the process type, oxygen for aerobic and carbon dioxide for anaerobic methane fermentation, for example. The electron donors are again categorised in organic donors (heterotrophic) and inorganic donors (autotrophic). This indicates that it will be possible to describe alkalinity yield coefficients for each of these categories, which simplifies the matter, making the use of the alkalinity yield coefficient practical.

**The True alkalinity yield**

The alkalinity yield is very similar to the growth yield which is dependent on the ratio of electron flow and the microorganism species, with a true growth yield specified for each species and growth environment (Grady *et al*. 1999) (making the true growth yield very specific). A **True alkalinity yield, $Y_{TALK}$** , can similarly be defined as the alkalinity yield without maintenance energy and by using Eq. 19, can be defined in terms of the true growth yield, Y, giving the theoretical True alkalinity yield:

$$Y_{TALK} = 1 - Y \,.\, 8/20$$

The correlation between $Y_{TALK}$ and $Y_{ALK}$ can be derived as follows. Considering the overall reaction, R (Eq. 18), with d, a, and c, the alkalinity production per unit of substrate consumed (donor), for the electron donor, electron acceptor and cell synthesis, respectively. Then the overall alkalinity production or yield is:

$$\begin{aligned} Y_{ALK} &= d + f_e a + f_s c \\ &= d + (1 - f_s)\, a + f_s c \qquad (f_e + f_s = 1) \text{ (McCarty 1975)} \\ &= d + a + f_s\,(c - a) \\ &= x + f_s y \\ &= x + Y_{obs}\, y \end{aligned} \qquad (20)$$

with $x = a + d$: the alkalinity production by the electron acceptor plus that of the electron donor, per unit substrate (donor) consumed in mole / mole.

$y = c - a$: the alkalinity production by cell synthesis minus that of the electron

acceptor, per unit substrate (donor) consumed in mole / mole.

$f_s = Y_{obs}$: for $Y_{obs}$ on a COD / COD basis.

$Y_{obs}$ is replaced by Y to determine $Y_{TALK}$ and therefore:

$$Y_{TALK} = x + Yy \qquad (21)$$

substituting Eq. 7 for $Y_{obs}$ in Eq. 20 gives:

$$Y_{ALK} = x + Yy \ / \ (1 + b\tau)$$

rearrangement to define Y by both equations and equalising them gives a general equation for $Y_{ALK}$:

$$(Y_{TALK} - x) / y = (Y_{ALK} - x)(1 + b\tau)$$

$$\therefore \quad Y_{ALK} = (Y_{TALK} - x) / (1 + b\tau) + x$$

$$= (Y_{TALK} + xb\tau) \ / \ (1 + b\tau) \qquad (22)$$

with x as defined above that can be determined from the half reactions as constructed by McCarty (1975).

The theoretical $Y_{TALK}$ may be calculated using Eq. 21 with Y known or with calculating Y by a method described by McCarty (1975), with dependence on the substrate used.

**Accuracy in using the theoretical alkalinity yield**

The change in the solution alkalinity as defined by Loewenthal *et al*. (1991), will depend on the change in the subsystem alkalinities. The accuracy in using the theoretical alkalinity yield will depend on the contribution of the considered substrate (as a subsystem) to the change in the solution alkalinity. **The bigger the contribution to the change in alkalinity by subsystems, other than the substrate subsystem, the bigger the inaccuracy will be in using the theoretical alkalinity yield**. An anaerobic process is an example in which the carbonate subsystem may play a major role in the alkalinity of a closed system with only limited $CO_2$ stripping and a significant increase in $CO_2$ partial pressure (Pretorius 1995). **These differences may be considered and added to the**

**theoretical yield coefficient, or the proposed equilibrium chemistry may be used to accurately calculate the alkalinity change**.

Combining Eqs. 17 and 22 gives an equation for the pHauxostat in terms of $Y_{TALK}$:

$$i_s X_{COD} (Y_{TALK} + xb\tau) / Y = S_{ALK} - S_{ALK0} \quad (23)$$

**Conclusion**

The alkalinity yield coefficient relates to the change in the solution alkalinities between the substrate and reactor solutions, and is relative to the change in the substrate concentration. The yield may be represented by a theoretical alkalinity yield, determined by half reactions, if the change in the solution alkalinities is only associated with the change in the substrate. A True alkalinity yield can also be defined, similarly to the True growth yield.

## 2.7 Feed control-method

The difference in the solution alkalinities between the feed and reactor solutions, together with the rate in alkalinity generation, was said to be the driving force for the chemo-pHauxostat feed system. For the bio-pHauxostat the alkalinity generation rate is given by Eq. 15:

$$r_{ALK} = (F/V) (i_s X_{COD} Y_{ALK} / Y_{obs})$$

The feed rate, F, can be expressed as:

$$F = r_{ALK} V / (i_s X_{COD} Y_{ALK} / Y_{obs})$$

substituting $(i_s X_{COD} Y_{ALK} / Y_{obs})$ with Eq. 16 gives:

$$F = r_{ALK} V / (S_{ALK} - S_{ALK0}) \quad (24)$$

Eq. 24 defines the feed rate and indicates the aspects determining the feed rate. The equation is similar to Eq. 12 for the chemo-pHauxostat. The equation indicates that **the feed rate is determined by the rate in alkalinity generation and the difference in the solution alkalinities**.

This confirms the conclusion drawn for the chemo-pHauxostat, which is also true for the bio-pHauxostat.

**The difference in solution alkalinities**

The solution alkalinity is the sum of the subsystem alkalinities, which is described above and does not need further explanation. The difference in the solution alkalinities between the feed and the reactor solutions relates to the difference in the species concentrations of the subsystems, or the degree of subsystem species removed. The more removed, the bigger the difference in alkalinity[1]. The difference in alkalinity is however determined by the difference in the pH between the substrate and reactor solutions, and the specific subsystem species removed by the bioprocess. The substrate pH may be changed by base addition, and the set point pH by selection. This will determine the required degree of subsystem species to be removed. **The difference in the solution alkalinity is therefore controllable and controls the degree of substrate removed**.

**The alkalinity generation rate**

The rate in alkalinity generation is according to Eq. 14 related to the rate of substrate consumption. Eq. 2 defines the rate of substrate consumption, which is related to the growth rate $\mu$, the specific growth rate coefficient. Combining Eqs. 2 and 14 gives:

$$r_{ALK} = \mu i_s X_{COD} Y_{ALK} / Y_{obs} \qquad (25)$$

indicating that **the rate in alkalinity change is determined by the growth rate**. The growth rate for the bio-pHauxostat is, as mentioned in Section 2.2 above, controlled via the pH and influenced by equilibrium chemistry that controls the GLN.

## 2.8 Conclusions for the bio-pHauxostat

- The pH is the controlled Output variable, with the feed rate the manipulated Input variable.

- The pH set point of the pHauxostat will determine the chemical species concentrations of the weak acid/base subsystems for the given substrate composition, and relates to the buffer

[1] *Vide* Fig. 2.4 p33

capacity of the reactor solution. This will determine the GLN concentration and will control the growth rate in the bio-pHauxostat.

- The reactor solution can be characterised by utilising solution and subsystem alkalinities in calculations considering the substrate and reactor solutions. This will enable the calculation of the GLN concentration.

- Alkalinity is a parameter that takes the influenced on pH into account and can be used to model the pHauxostat. It relates to the reference species used to define the alkalinity.

- An alkalinity yield coefficient can be defined, linking biological growth with water chemistry.

- A theoretical alkalinity yield coefficient can be calculated using oxidation-reduction half reactions, expressed by Eqs. 20 and 22, respectively:

$$Y_{ALK} = x + Y_{obs}y$$
$$Y_{ALK} = (Y_{TALK} + xb\tau) / (1 + b\tau)$$

- The bio-pHauxostat feed rate is determined and controlled by the difference in the solution alkalinities between the substrate and reactor solutions and the alkalinity generation rate, expressed by Eq. 24:

$$F = r_{ALK} V / (S_{ALK} - S_{ALK0})$$

with the difference in solution alkalinities determined by the pH set point and the substrate composition. The alkalinity generation rate is dependent on the culture growth rate.

- The bio-pHauxostat can be modelled by Eqs. 16 and 17, respectively:

$$i_s X_{COD} Y_{ALK} / Y_{obs} = S_{ALK} - S_{ALK0}$$
$$i_s X_{COD} Y_{ALK} (1 + b\tau) / Y = S_{ALK} - S_{ALK0}$$

# 3. THE pHAUXOSTAT

*In this section the pHauxostat is considered in general, different types identified, categorised and control methods proposed.*

## 3.1 The pHauxostat in general

### Defining pHauxostats

A definition for the pHauxostat will evolve around the control configuration and the use of the variables within the process. The control configuration for the pHauxostat was explained in Chapter I as a Closed loop, Feedback and Self-regulating control. This control configuration describes a number of different types of applications, for example the Turbidostat, the Nutristat and also the pHauxostat, and is not specific enough for defining the pHauxostat. These self-regulating systems utilise different Output variables as the controlled parameter with the feed as the Manipulated Input variable. The pHauxostat is distinguished by the use of the pH as the controlled Output variable with the feed rate and/or the substrate concentration as the Manipulated Input variables. An appropriate definition would be; "A self-regulated control technique in biotechnology, whereby the pH is used as the controlled output variable, and the feed (rate and/or concentration) the manipulated input variable." The published studies and applications mentioned in Chapter I are all describable by this definition.

### Different applications

Analysing the completed studies it is noticed that different applications for the pHauxostat are possible and that different nutrients can be used as the growth limiting nutrient. Martin & Hempfling (1976) used the technique for aerobic and anaerobic growth. Rice & Hempfling (1985) operated the pHauxostat with succinate, sulfate and phosphate the GLN. Duetz *et al.* (1991) applied growth under non-limiting concentrations in a pHauxostat. Rice & Hempfling (1978) used oxygen as the GLN, a gaseous nutrient and not part of the self-controlled Manipulated Input variable. Sowers *et al*. (1984) and Pretorius (1995) used SCFA as substrate with the pH increasing through bio-consumption. Driessen *et al*. (1977) and MacBean *et al*. (1979) on the other hand operated pHauxostats with the pH change brought about by lactic acid production, during milk fermentation.

From these applications it is concluded that the GLN can be the carbon source or any other nutrient and can be part of the manipulated feed stream or not. Another important difference in application is in how the weak acid/base subsystems are influenced by growth. Two possibilities may be distinguished in that the subsystems may either be influenced by removal, or by addition of subsystem species. The difference being that the weak acid/base subsystem species may either act as substrate consumed or as products produced or both and thereby influence the equilibrium chemistry and pH.

Considering all these differences it is possible to categorise pHauxostats into different types.

**Categorising pHauxostats**

The conceptualised bio-pHauxostat is one in which the GLN is part of a weak acid/base subsystem. The added base and the pH set point determine the GLN concentration. The GLN concentration is inter related and a function of the pH. This is defined as a Category A pHauxostat; a pHauxostat of which the GLN concentration, S, is a function of the set point pH, therefore: $S = f(pH)$. A Category B pHauxostat is by analogy a pHauxostat of which the GLN concentration is not a function of the set point pH, therefore $S \neq f(pH)$.

The focus for categorising pHauxostats is set on the growth limiting nutrient and its interaction with the weak acid/base subsystems. The parameter determining the growth rate and its correlation with the parameter used as control is thereby taken into consideration.

A further sub-division may be done on whether the GLN is part of the manipulated feed or not. This gives two main Categories; Category A and B, with sub-divisions; A1 and A2, and B1 and B2. A1 and B1 defined as pHauxostats of which the GLN is part of the manipulated feed and A2 and B2 pHauxostats of which the GLN is not part of the manipulated feed.

## 3.2 Category A pHauxostats

**The GLN**

Category A pHauxostats are pHauxostats of which the growth limiting nutrient concentration is a function of the pH; $S = f(pH)$. This definition implies that the GLN has to be part of one of the

weak acid/base subsystems and at such a concentration that it influences the pH of the reactor solution. The GLN concentration is thereby controlled through equilibrium chemistry and can be manipulated by the substrate composition and the pH set point. Equilibrium chemistry can be used to characterise the system, to calculate the GLN concentration and to determine the desired operating point.

The GLN may strictly speaking be any nutrient required by the culture for growth that is part of a subsystem influencing the pH. Practically however it is necessary that the change in the nutrient concentration be big enough to exert enough influence on the feed system to make control possible. The macronutrients, nitrogen and phosphorus are required in much less quantities for heterotrophic growth compared to carbon, and have a very low and limited concentration range when acting as the GLN (Grady *et al*. 1999). At these concentrations of less than a few milligrams per litre, its influence on the pH is limited and will be negligible in a slightly buffered system. The GLN will therefore normally be the carbon and energy source to be able to cause enough change in the solution alkalinity to make the technique work. It also follows that the carbon and energy source will normally be an organic acid as the weak acid/base of concern, as in the case of the demonstrated bio-pHauxostat. Exceptions may be autotrophs, for example *Nitrosomonas,* in a nitrification process with a very low buffer capacity.

**Subdividing Category A pHauxostats**

The subdivision of Category A pHauxostats into Categories A1 and A2 pHauxostats are probably more academic than practical. With the GLN normally the carbon and energy source it means that the liquid volume and therefore the manipulated feed, will normally contain the GLN, resulting in a Category A1 pHauxostat. In the case of a Category A2 pHauxostat, the manipulated feed will need to be dilution water with the carbon source being added by another method, for example, a manually controlled feed stream. This type set-up is possible as a laboratory technique but unlikely in full scale application.

**Alkalinity generation**

Considering that the pHauxostat technique is interrelated with alkalinity generation and that alkalinity generation can be brought about by substrate consumption and/or product production, it implies that for Category A pHauxostats alkalinity generation must be brought about by substrate

consumption to conform to the definition of S = f(pH). It may however simultaneously be brought about by products produced. This may be uncommon but possible in for example tightly controlled fermentation processes. Anaerobic treatment of an organic acid stream may also be such an application. Alkalinity is generated by acid removal but also influenced by the carbon dioxide produced in a system of high carbon dioxide partial pressure, influencing the pH (Pretorius 1994). The partial pressure is however externally controlled and it is arguable whether it can be classified as such an application or not.

To conclude, alkalinity will always be generated by substrate consumption for a Category A pHauxostat, but may also be generated by both, substrate consumption and product production.

**Control methodology**

Category A pHauxostats are controlled by the following sequence: The given substrate composition and pH set point determines the GLN concentration and the difference in the alkalinity between the substrate and reactor solutions. This implies that the growth rate is set and fixed and thereby also the HRT and the feed rate (Eq. 6). With the feed rate fixed and a fixed difference in alkalinity, it means that the rate in alkalinity change is fixed (Eq. 24). Now depending on the alkalinity yield coefficient, a certain amount of biomass is required to bring the given rate in alkalinity change about, whereby the biomass concentration is determined (Eq. 15). It may be presented in a flow diagram :

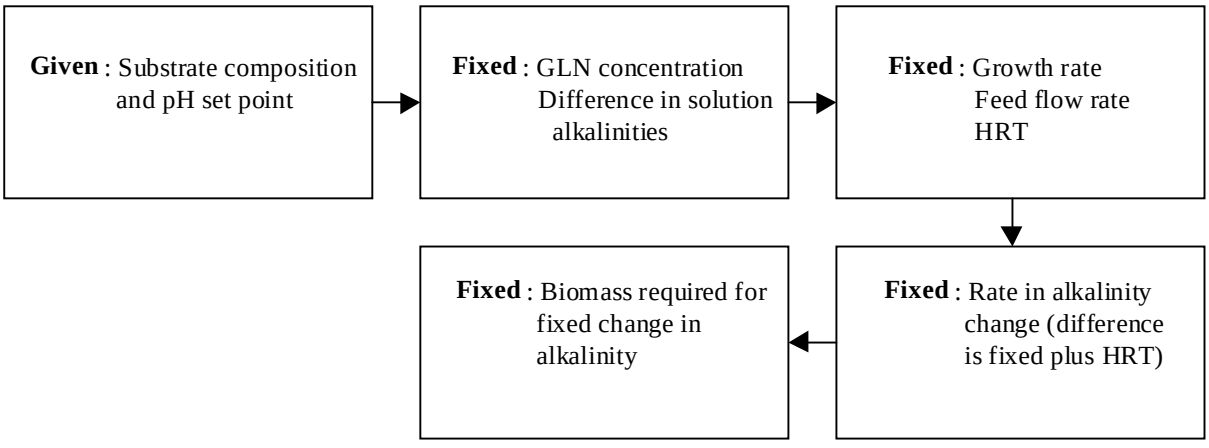


FIG. 2.5 - Control methodology : Category A pHauxostats

In summary it can be said that the substrate composition and the pH set point determine the growth rate, and the biomass concentration is determined by the alkalinity yield coefficient and the difference in alkalinity. The process can be manipulated by changing the GLN concentration and the difference in alkalinity between the two solutions by manipulation of the pH set point and the substrate composition.

Referring to the definition of a Nutristat by Edwards *et al*. (1972) whereby the GLN concentration is controlled and kept constant by the control technique, it follows that a Category A pHauxostat may also be described by this definition and is thus a Nutristat.

## 3.3 Category B pHauxostats

### The GLN

Category B pHauxostats are pHauxostats of which the GLN is not influenced by the solution pH, therefore S is not a function of the set point pH; $S \neq f(pH)$. This means that the GLN is not part of a weak acid / base subsystem or is of such low concentration that it does not have any influence on pH. An example is the conceptual bio-pHauxostat but with nitrogen or phosphorus the GLN. With its concentration so low and with a limited range as the GLN, it will not have any significant influence on the pH. The nitrogen or phosphorus may either be part of the substrate, at a concentration low enough to result in growth limitation, or it may be supplied via another feed stream with external control. These applications can respectively be categorised as B1 and B2 pHauxostats.

The GLN may be any nutrient required for growth, including a gaseous nutrient, for example oxygen (Rice & Hempfling 1978; Gottschal 1990). The pHauxostat technique was shown to be ideal in studies of oxygen limitation (Gottschal 1990). The substrate is controlled via the pH-controller while oxygen is supplied as part of the air supply with a External control, for example manually. The air supply may be decreased until oxygen limitation results and thereby control the growth rate.

Category B pHauxostats will be explained by using oxygen as the growth limiting nutrient which does not form part of a weak acid/base subsystem and is not supplied as part of the manipulated

feed stream. The GLN is supplied via a External control and is categorised as a Category B2 pHauxostat.

**Control methodology**

A decrease in the oxygen supply rate will decrease the rate of substrate consumed and the rate of alkalinity generation and will slow the feed and growth rate. The control method is thus different from that of a Category A pHauxostat, where growth rate is controlled via equilibrium chemistry.

Category B pHauxostats is controlled by the following sequence: The supply rate (load rate) of the GLN, controls the rate of substrate consumption (Eq. A7) and thereby the amount of biomass required (for this consumption). It also determines the rate of alkalinity generation which is dependent on the alkalinity yield coefficient (Eq. 14) and depending on the difference in alkalinity, controls the feed rate (Eq. 24). The feed rate controls the growth rate and implies a corresponding GLN concentration in the reactor (Eq. 3). It may be presented in a flow diagram :

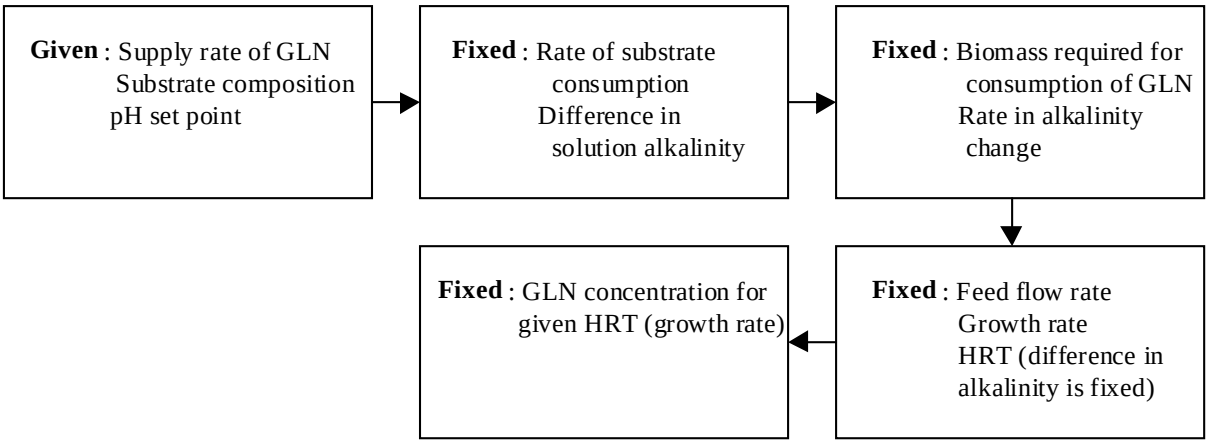


FIG. 2.6 - Control methodology : Category B pHauxostats

In summary it can be said that the biomass concentration and the alkalinity generation rate, are determined by the supply rate of the GLN and the alkalinity yield coefficient. The growth rate and the corresponding GLN concentration are determined by the difference in alkalinity and the alkalinity generation rate. The process can be manipulated by changing the substrate composition and/or the pH set point, whereby the difference in alkalinity is changed. This will influence the

growth rate and the GLN concentration. The biomass concentration is mainly determined by the feed rate of the GLN.

**Category A versus Category B pHauxostats**

The control mechanism for the two categories is in principle the same but works in opposite directions. Category A starts by control of the GLN concentration and results in the amount of GLN consumed. Category B starts with the control of the amount of GLN consumed and results in the concentration of the GLN. The controlled Output variable stayed the pH and the Manipulated Input variable the feed flow rate, but the GLN concentration for Category B pHauxostats is not directly controlled by the controlled Output variable (pH). This implies that a Category B pHauxostat can not be defined as a Nutristat. A further difference is that alkalinity generation for Category B pHauxostats may be brought about by any combination of substrate consumption and product production. Alkalinity generation may be brought about by only product production, whereas for Category A pHauxostats substrate consumption always needs to be part of alkalinity generation.

# CHAPTER III - VERIFICATION

*The conclusions made in the previous chapter are verified through laboratory testing in this chapter. Traditional equilibrium chemistry with developed equations for this application was verified in Appendix B and is applied here. The feed method is firstly considered, applied in a chemo-pHauxostat, where after Category A and B pHauxostat are investigated.*

## 1. THE FEED METHOD : CHEMO-pHAUXOSTAT

### 1.1 Purpose of laboratory test work

This test work considers the solution and subsystem alkalinities defined in Chapter II, test the proposed theory whether the flow rate is determined by the difference in alkalinity and the alkalinity generation rate (Eq. 12), and verify whether the control technique keeps the solution alkalinity constant.

### 1.2 Experimental methods

A pHauxostat as shown in Fig. 2.1[1] was set up. Peristaltic pumps were used with a Hanna pH502523 pH controller with PID control and analog output and a Hanna HI2911 B/5 pH probe. The feed pump, a Watson-Marlow 313U pump with analog input, was interlinked with the pH controller. The NaOH addition pump, a Gilson Miniplus 3, was manually set, adding NaOH at a 0,5N concentration. Feed was made up with freshly distilled water, acetic acid, ammonium chloride and phosphoric acid to concentrations of 501, 100 and 50 mg/*l*, as HAc, N and P, respectively. The same approximate concentration ratios were used as for the equilibrium chemistry test work reported in Appendix B. A one litre Erlenmeyer flask was used as reactor and the controller set to a pH of 5,15.

The reactor was filled with feed, aerated, pH controller and feed pump activated and the NaOH feed started. The flow rates of NaOH and feed addition were volumetrically and cumulatively measured over time, and together with pH and temperature recorded. The NaOH addition was kept constant

[1] *Vide* Chapter II p16

for approximately one hydraulic retention time and recorded as Run 1. The NaOH addition was thereafter increased for another retention time and recorded as Run 2.

### 1.3 Results and Explanation

The averaged results are shown in Table 3.1 with calculated and stabilised mass addition rates shown in Table 3.2.

TABLE 3.1 - Average measured flow rates

| Run | Time (min) | NaOH (m*l*/min) | Feed (m*l*/min) | pH |
|---|---|---|---|---|
| 1 | 0 – 8,5<br>8,5 – 12<br>12 → 1xHRT | 0,119 | 6,07<br>6,80<br>6,88 | 5,17 / 5,18<br>5,17 / 5,18<br>5,18 |
| 2 | 0 – 3,3<br>3,3 – 24<br>24 – 32<br>32 – 48<br>48 → 1xHRT | 0,221 | 7,90<br>8,63<br>16,66<br>14,75<br>12,64 | 5,18 / 5,20<br>5,20 / 5,22<br>5,23 / 5,29<br>5,29 / 5,24<br>5,24 |

The pH increased with the start of NaOH addition with a corresponding slow increase in feed rate. The feed flow rate stabilised after a few minutes at a pH slightly higher than the set point pH (5,15). This difference is ascribed to the PID control system and its setting and can be adjusted to decrease the difference. No changes were observed thereafter within the one HRT of approximately two hours for Run 1.

The pH slowly increased with commencement of Run 2, taking longer to stabilise on a pH slightly higher than for Run 1. This difference in pH is also attributed to the PID control system and its

setting. The pH and flow rate was stable for the rest of the one HRT of approximately one and a quarter hour.

TABLE 3.2 - Stabilised flow rates and calculated mass addition rates

| Run | Addition | Flow rate (m*l*/h) | Mass rate (mg/h) |
|---|---|---|---|
| 1 | $NaOH_0$ | 7,13 | 142,6 |
| | Feed | 412,9 | - |
| | N | - | 41,4 |
| | P | - | 20,5 |
| | Hac | - | 206,7 |
| | Feed + NaOH | 420,0 | - |
| 2 | $NaOH_0$ | 13,23 | 264,6 |
| | Feed | 758,6 | - |
| | N | - | 76,0 |
| | P | - | 37,6 |
| | Hac | - | 379,8 |
| | Feed + NaOH | 771,9 | - |

**Ratio of feed to NaOH at constant pH**

The ratio of the mass addition rate of the feed species versus the NaOH addition rate in Table 3.2 and the titration concentration ratios in Table B3[1] (solution 4) (Appendix B), are in the same order for approximately the same pH's. The calculated ratios of HAc : NaOH are 1,45:1 for Run 1 and 1,44:1 for Run 2 versus 1,39:1 for solution 4 (aerated). The pH of solution 4 was 5,51 measured, and 5,58 calculated, versus the pH for Run 1, 5,18 and for Run 2, 5,24.

[1] *Vide* Appendix B p123

The results confirm the neutralising chemical reaction, taking place in the reactor that is similar to a titration of the feed with NaOH. The equilibrium pH values for Run 1 and 2 can be calculated for comparison and are given in Table 3.3.

TABLE 3.3 - Calculated equilibrium pH's for Runs 1 and 2

| Run | Solution concentrations (mg/*l*) | Temp. °C | I (mol/l) | pH measured | pH calculated |
|---|---|---|---|---|---|
| 1 | P/N/HAc/NaOH: 48,8 / 98,5 / 492,1 / 339,6 | 25,4 | 0,0155 | 5,18 | 5,42 |
| 2 | P/N/HAc/NaOH: 48,7 / 98,5 / 492,0 / 342,8 | 25,2 | 0,0156 | 5,24 | 5,45 |

The calculated pH values differ with 0,24 and 0,21 units for Run 1 and 2, respectively, indicating close approximation. The bigger differences compared to the differences in Table B3 are attributed to the accuracy of the volumetric measurement of the flow rates.

**Solution and subsystem alkalinities**

Using equilibrium chemistry and the solution concentrations in Table 3.3, the subsystem and solution alkalinities were calculated and summarised in Table 3.4. Considering the solution alkalinities ($S_{ALK0}$ and $S_{ALK}$), it is seen that the alkalinities for Run 1 and 2 are nearly the same. These should in fact be exactly the same, considering that the pH set point and feed composition stayed the same. The only reason for the differences are the small differences in the solution concentrations and in the stabilised pH values. It confirms that the pHauxostat control technique keeps the reactor alkalinity constant.

**Feed flow rate**

The feed flow rate was calculated as the feed plus the NaOH flow rate, added together. This is in line with the assumption made in deriving Eq. 12, that alkalinity is generated and not added. The values for alkalinity generation, $NaOH_o$, were calculated using the mass addition rates in Table 3.2. These were used in Eq. 12 plus the solution alkalinities in Table 3.4, to calculate the theoretical flow rates. The results are shown in Table 3.5 and compared to the measured total feed rates from

TABLE 3.4 - System and solution alkalinities (mol/*l*)

| Alkalinity | Run 1 | | Run 2 | |
|---|---|---|---|---|
| | Feed | Reactor | Feed | Reactor |
| pH | 2,842 | 5,421 | 2,843 | 5,453 |
| Alk $H_2CO_3^*$ | $3{,}08 \times 10^{-9}$ | $1{,}21 \times 10^{-6}$ | $3{,}10 \times 10^{-9}$ | $1{,}31 \times 10^{-6}$ |
| Alk $H_2O$ | -0,001574 | $-4{,}30 \times 10^{-6}$ | -0,001576 | $-3{,}99 \times 10^{-6}$ |
| Alk HAc | -0,000108 | 0,006880 | 0,000108 | 0,006962 |
| Alk $H_3PO_4$ | 0,001329 | 0,001612 | 0,001327 | 0,001612 |
| Alk $NH_4^+$ | $3{,}10 \times 10^{-9}$ | $1{,}21 \times 10^{-6}$ | $3{,}06 \times 10^{-9}$ | $1{,}29 \times 10^{-6}$ |
| $S_{ALK0}$ | -0,000137 | - | -0,000140 | - |
| $S_{ALK}$ | - | 0,008490 | - | 0,008572 |
| $S_{ALK} - S_{ALK0}$ | | 0,008627 | | 0,008712 |

Table 3.2. The calculated and measured flow rates compare well with differences of 1,6 %. This indicates the validity of Eq. 12.

TABLE 3.5 - Calculated feed flow rates compared to measured rates

| Run | $NaOH_o$ (mol/h) | $F_o$ calculated (m*l*/h) | $F_o$ measured (m*l*/h) | Difference % per measured |
|---|---|---|---|---|
| 1 | 0,003566 | 413,3 | 420,0 | 1,6% |
| 2 | 0,006616 | 759,4 | 771,9 | 1,6% |

With the solution alkalinities for Run 1 and 2 the same, the only difference between Run 1 and 2 is the rate in alkalinity generation (addition) that increased. To balance the increased rate in alkalinity generation with the "negative" feed alkalinity, and thereby keep the solution alkalinity in the reactor constant, the feed flow rate automatically increased. The alkalinity load rate was automatically increased, by increasing the flow rate, because the concentration of the feed alkalinity stayed the same. It is clear from this action that the difference in alkalinity between the feed and reactor solutions will influence the feed flow rate. It can be concluded that for the same alkalinity generation rate, the feed flow rate will respectively, increase or decrease with a decrease or increase in the difference in the solution alkalinities. This change in the difference in solution alkalinities may be brought about by a change in the buffer capacity of the feed and/or a change in the pH difference.

## 1.4 Conclusions

The test work confirmed that the control technique controls the pH but also the solution alkalinity, by keeping it constant. Eq. 12 was verified, indicating that the feed flow rate is determined by the difference in alkalinity between the feed and the reactor solutions (buffer capacity) in combination with the alkalinity generation rate. The conclusions are summarised below :

- The solution alkalinity of a mixture of weak acid/base subsystems is the sum of the subsystem alkalinities relative to its reference species and can be calculated using equilibrium chemistry.

- The pH and the solution alkalinity in the reactor are kept constant by the pHauxostat feed system.

- The apparent rate in alkalinity change and the difference in the solution alkalinities between the feed and the reactor determine the feed flow rate.

- The apparent rate in alkalinity change for the chemo-pHauxostat is the mass loading rate of base added to the reactor.

- The controlled Output variable for the pHauxostat is the pH with the manipulated Input variable the feed flow rate for a fixed feed composition.

# 2. THE pHAUXOSTAT

## 2.1 Purpose of test work

The test work was done to demonstrate a Category A and a Category B pHauxostat with the GLN respectively acetic acid and oxygen; to verify the proposed method of equilibrium chemistry, by using solution and subsystem alkalinities to characterise the solution in the bioreactor; to confirm the difference in the buffer capacity between the substrate and the reactor solutions; and to verify the proposed theory and alkalinity yield coefficient and the suggested control methodologies.

## 2.2 Experimental methods

The laboratory test work was done by completing two test runs; Test Run A: The effect of a change in the feed alkalinity; and Test Run B: The effect of a decrease in air supply.

### Substrate

Substrate similar to the feed solution for the chemo-pHauxostat was used. Acetic acid as carbon and energy source with ammonium chloride and phosphoric acid as the nitrogen and the phosphorus macronutrients, respectively. The macro- and micronutrient recipes are given in Tables 3.6 and 3.7, respectively. The substrate was made up in freshly distilled water resulting in a chemically defined substrate with all elements known. Chemicals of AR quality were used. Fresh substrate was made up for each test run, sterilised in an autoclave at 121°C for 20 minutes and stored in a feed drum at 4°C. The substrate was made-up to 10 g/l HAc for Test Run A and to 5 g/l for Test Run B.

### Culture

The yeast *Candida utilis* was isolated as dominant species in pre-test runs with the specified substrate as selection medium (Pretorius 1987). The Department of Microbiology and Biochemistry at the University of the Free State (RSA) did the identification of the culture.

The culture was subcultured on agar slants and used as inoculum in shake flasks. Shake flask medium was made up to 2 g/*l* HAc with corresponding nutrients, and sterilised in the flasks. The

TABLE 3.6 - Macronutrients

| Chemical | Macronutrient | Nutrient mg/10g HAc |
|---|---|---|
| $CH_3COOH$ | Carbon and energy | 10 g |
| $(NH_4)_2SO_4$ | S (+N) | 43 |
| $NH_4Cl$ | N | 291* |
| $H_3PO_4$ | P | 101 |
| KCl | K | 10 |
| $MgSO_4 .7H_2O$ | Mg | 7 |
| $CaCl_2 .2H_2O$ | Ca | 3 |
| $FeSO_4 .7H_2O$ | Fe | 0,5 |
| NaOH | Na | in excess |

* Nitrogen for both chemicals $(NH_4)_2SO_4$ and $NH_4Cl$.

TABLE 3.7 - Micronutrients*

| Chemical | Nutrient | Nutrient mg/10g HAc |
|---|---|---|
| $H_3BO_3$ | B | $1,24 \times 10^{-2}$ |
| $MnSO_4 .5H_2O$ | Mn | $2,41 \times 10^{-1}$ |
| $ZnCl_2$ | Zn | $2,01 \times 10^{-1}$ |
| $Co(NO_3)_2 .6H_2O$ | Co | $0,91 \times 10^{-1}$ |
| $MoO_3$ | Mo | $6,60 \times 10^{-2}$ |
| $CuSO_4 .5H_2O$ | Cu | 0,38 |
| $NiCl_2$ | Ni | $6 \times 10^{-3}$ |

* Micronutrients made-up as a combination from Nel, Britz & Lategan (1985) series I, and Du Preez & Van der Walt (1983).

flasks were shaked after inoculation for two to three days before transfer to the pHauxostat reactor. Each run was started with fresh inoculum and the procedure repeated.

**pHauxostat lay-out**

The lay-out is shown in Fig. 3.1. The substrate was stored in a cold room and transferred with a Watson-Marlow 313U peristaltic pump to the reactor. The pump, with analog input control, was controlled via a Hanna pH502523 pH controller with PID control and analog output. A Hanna HI2911 B/5 pH probe was used. The reactor was constructed using a 200mm diameter Perspex pipe with approximately 5 *l* working volume. Temperature control was done with a heat exchanger on the circumference of the reactor. Effluent was pumped from the reactor with a Gilson miniplus 3 peristaltic pump, keeping the reactor level constant. Filtered compressed air was used for aeration and measured with a Fischer&Porter rotameter. The air flow rate was manually set and the pressure measured with a water manometer. The reactor was open to the atmosphere. The reactor set-up was kept in a warm room at approximate 27 $^{o}$C. A photo print of the set-up is shown in Appendix D.

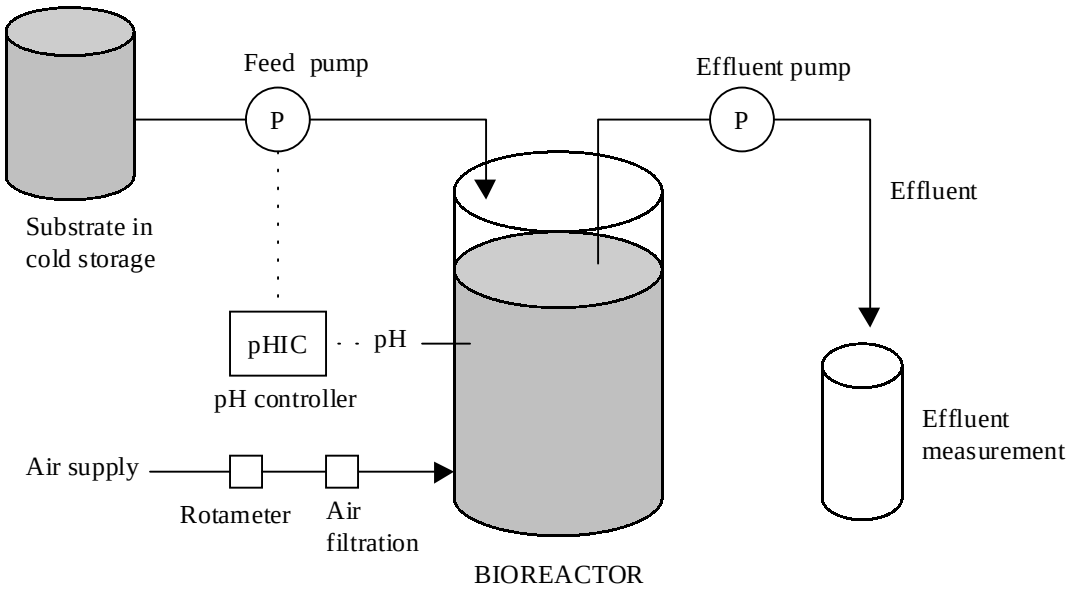


FIG. 3.1 - The pHauxostat lay-out

**Test runs**

**Test Run A** : Three runs were completed as part of Test Run A, by varying the NaOH concentration in the substrate, as per Table 3.8. The dissolved oxygen concentration was measured daily and kept above 2 mg/*l* not to be limiting (Grady *et al*. 1999).

TABLE 3.8 - NaOH concentration for Test Run A : Runs A1, A2 and A3

| Run | NaOH added to substrate (mg/l) |
|---|---|
| A1 | 627 |
| A2 | 878 |
| A3 | 1574 |

**Test Run B** : Three test runs were completed as part of Test Run B. The air flow rate was decreased for the second and third run to induce oxygen limitation. The air flow rates are given in Table 3.9. NaOH addition to the substrate was kept constant at 501 mg/*l* for the three runs.

TABLE 3.9 - Air flow rates for Test Run B : Runs B1, B2 and B3 at 101,3 kPa and 0°C

| Run | Air flow rate in *l*/min | Air flow rate in *l*/*l*.min* |
|---|---|---|
| B1 | 3,4 | 0,68 |
| B2 | 0,7 | 0,14 |
| B3 | 0,36 | 0,07 |

* *l* air per minute per *l* reactor volume.

**Monitoring and analyses**

Each run was monitored until steady state operation was established. This was evaluated by monitoring the change in the feed flow rate, steady state was assumed when no steady change in feed flow rate was noticed and the average flow rate stayed constant over a 24-hour period (approx. two HRT's). The effluent flow rate was continuously volumetrically measured and assumed to be equivalent to the feed rate. Microscopic observation was frequently done by phase contrast microscopy to monitor bacterial contamination and for physiological observations of growth. Samples were routinely taken and analysed for biomass concentration and for filtered; COD, SCFA, ammonia and phosphate. Three final samples were taken, evenly spaced, over a twelve-hour period after steady state operation was established. These samples were additionally tested for potassium, magnesium and calcium. All analyses were done as per Standard Methods (1995) except for SCFA that was done on a 5 pH point titration method (WRC 1992).

**Inoculation**

All apparatus was sterilised in an autoclave at 121°C for 20 minutes, or disinfected for more than 24 hours with calcium hypochlorite, before start-up. The pH controller was calibrated and the set point set to a pH of 5,50. Aeration was started and the contents of the flasks with the *Candida utilis* culture transferred to the reactor. The contents of one 250 mg chloramphenicol capsule was added. The flasks' contents were approximately 2,4 *l*. The feed pump was switched on and the process operated as a fed-batch pHauxostat until the set reactor level was reached. The effluent pump was switched on and the process thereafter operated as a continuous pHauxostat.

**Calculations using solution and subsystem alkalinities**

Applying the dosing method from Loewenthal *et al.* (1991) and using HAc as the unknown to be compared against the test results, the following procedure was followed. The substrate was characterised using equilibrium chemistry as per Appendix B, and the solution alkalinities together with the subsystem alkalinities calculated for the selected reference species. The total species concentrations as made-up (Tables 3.6 and 3.8) were used. The solution alkalinities for the substrate and reactor were assumed to be the same, with the total species concentrations and pH known for the reactor solution by measurement (test results). The subsystem alkalinities were calculated, except for HAc. The Alk HAc was now calculated by the difference between the

solution alkalinity and the summed subsystem alkalinities. Once the Alk HAc was known, the reactor solution can be characterised.

**Calculating $f_s$**

Working in COD units is not necessary but was used in the equation development to be compatible with the generally accepted acronyms and units in the wastewater field. The measured alkalinity yield, $Y_{ALK}(m)$, was however for simplicity calculated by using biomass as measured in TSS/*l* and $Y_{obs}$ in units of biomass per HAc consumed, with $i_s$ in Eq. 16 changed to moles per g HAc (inverse of molar mass of HAc = $^1/_{60}$ mol/g). $f_s$ was calculated on a COD/COD basis using laboratory measured values for the biomass (0,81 VSS/TSS and 1,34 g COD/g VSS) and the difference in COD between in and out. The COD out was calculated by using $S_A$ out, multiplied by the theoretical COD : HAc ratio (64:60). The calculated effluent COD values will be less than the measured values. The measured values will include COD from cell products that were synthesised from substrate consumption with a corresponding alkalinity generation (Grady *et al.* 1999). The use of the measured effluent COD will therefore under estimate $Y_{ALK}$ , and is the change in HAc (the substrate), converted to COD, the correct method to use.

## 2.3 Results and Explanation

**General experience**

A number of tests runs were completed that was unsuccessful or incomplete due to problems experienced with equipment, instruments, start-up, operation and analyses. After these teething problems and with gained experience, the pHauxostat technique proved to be very reliable and operated without any interference. The only aspect that required attention was the calibration of the pH controller, that tended to drift in one direction within the first day or two before it stabilised. The most practical solution to the problem was to determine the difference in the pH with a calibrated portable pH meter and change the set point pH, to compensate for the difference. The actual pH was then noted as the pH reading from the controller plus or minus the off set value.

Substrate feeding during the fed-batch operation started slowly and increased with time. After continuous operation commenced, the flow rate stabilised to near constant within 24 hours, or approximately two retention times. Thereafter a slow increase or decrease was noticed until steady

state was reached. It can be assumed that the reactor contents followed a washout curve for the different chemical species from the flasks' contents (Levenspiel 1999). The flasks' content concentrations were selected as close as possible to the expected steady state concentrations to decrease the stabilisation time. Steady state was normally experienced within three days and the test runs completed within four days. Bacterial contamination was noticed after four to five days of operation where after a drastic increase resulted within a day or two.

The reactor temperature was controlled at ± 0,5°C from the set point temperature for Test Run A, and after improvement, controlled at 28,6°C $\pm$ 0,01°C for Test Run B. The pH had a drift of approximately 0,12 pH units for Test Run A, and after improvement was controlled at 5,52 $\pm$ 0,01 pH, for Test Run B.

**Steady state results**

The steady state results are given in Tables 3.10 and 3.11 for Tests Runs A and B, respectively. Complete analyses for Run B2 was not done. The potassium, magnesium and calcium concentrations in the reactor solutions were measured to be between 0,5 and 2 mg/*l*.

TABLE 3.10 - Steady state results for Test Run A – varying NaOH concentration

| Run | Temp. °C | $\tau$ (h) | X g/*l* | COD mg/*l* | $S_A$ mg/*l* | $NH_3$-N mg/*l* | $PO_4$-P mg/*l* |
|---|---|---|---|---|---|---|---|
| A1 | 27,5 | 14,1 | 2,00 | 228 | 80 | 89 | 40 |
| A2 | 27,5 | 11,8 | 2,66 | 555 | 340 | 110 | 46 |
| A3 | 28,0 | 10,4 | 2,30 | 1785 | 1480 | 112 | 49 |

The air flow rate was decreased by a few factors for Runs B2 and B3 to lower the dissolved oxygen (DO) concentration ($S_o$). This decreased the mixing intensity and resulted in a non-uniform DO concentration in the reactor. The DO was measured in different zones within the reactor and a weighted average concentration calculated.

TABLE 3.11 - Steady state results for Test Run B – varying aeration rate

| Run | τ (h) | X (g/*l*) | DO (mg/*l*) | COD (mg/*l*) | $S_A$ (mg/*l*) | $NH_3$-N (mg/*l*) | $PO_4$-P (mg/*l*) |
|---|---|---|---|---|---|---|---|
| B1 | 10,14 | 1,29 | 4,2 | 389 | 253 | 52 | 16 |
| B2 | 11,59 | 1,31 | 0,29* | - | - | - | - |
| B3 | 16,24 | 1,20 | 0,12* | 525 | 343 | 70 | 27 |

* weighted average

**Acetic acid versus COD**

Tables 3.10 and 3.11 include the average COD and HAc ($S_A$) values for each Run. The concentration ratios for HAc : COD can be calculated. The ratios for Test Run A are 0,35; 0,61 and 0,83 for Runs A1, A2 and A3, respectively. The ratio decreased with an increase in HRT/SRT. The theoretical ratio for HAc : COD is 0,94 : 1. This indicates that the reactor solution contains a relative high concentration of other organic compounds, besides HAc. According to Grady *et al.* (1999) these may include microbial products and cell material from cell lysis. It implies that differences will result in calculations by using COD versus HAc as the GLN or substrate. HAc is used in calculations in this study.

**The growth limiting nutrient**

i) Test Run A:

The results indicate an increase in $S_A$ and in the growth rate (decrease in HRT) with an increase in the NaOH concentration in the substrate. The increase in growth rate with an increase in $S_A$ while the other macronutrient concentrations stayed high and relative constant, indicate that HAc was the growth limiting nutrient.

*Candida utilis* is known to utilise HAc as carbon source and researchers Defrance *et al.* (1996) and Šestáková (1979) reported studies utilising this culture with HAc as the GLN. Interesting is that

*Candida utilis* has been used as a single cell protein (Martin *et al.* 1993; Defrance *et al.* 1996; Blanch & Clark 1997).

With HAc the GLN but also part of a weak acid/base subsystem, and the $S_A$ shown to be controlled by the base added, it follows that the growth rate is controlled by the amount of base added to the substrate, as demonstrated (Table 3.8 and 3.10). The control technique keeps the pH constant and thus the GLN. The growth rate is therefore automatically controlled by the control technique, implying a Self-regulated control which is also a Feedback control and by definition a Nutristat (because the GLN is controlled).

ii) Test Run B:

With nitrogen, phosphorus, DO, potassium, magnesium and calcium concentrations high and the HAc concentration similar to that of Test Run A, it can be assumed that HAc was the GLN for Run B1. The air supply was decreased and the DO concentration monitored for Runs B2 and B3. The substrate feed rate started to decrease at a DO concentration of less than 1 mg/*l*. The HRT increased for Runs B2 and B3 with only the DO concentration decreasing (Table 3.11), indicating oxygen limitation. This implies a change in the GLN from HAc to oxygen and also from a Category A to a Category B pHauxostat. It demonstrates a pHauxostat with the GLN (oxygen) not part of a weak acid/base subsystem and therefore with no correlation with the pH, and also not part of the manipulated substrate feed stream, implying a Category B2 pHauxostat.

The GLN concentration and the growth rate were changed by only changing the supply of the GLN, confirming the proposed control methodology for Category B pHauxostats. It also indicates that it can not be defined as a Nutristat because the control technique does not directly control the GLN.

**Equilibrium chemistry of the bio-process**

i) Equilibrium chemistry with a proton balance

The HAc concentration for Test Run A increased with an increase in NaOH concentration while the ammonia and phosphate increased slightly. Using the HAc, nitrogen and phosphorus concentrations from Table 3.10 together with the added NaOH from Table 3.8, the equilibrium pH was calculated for each run, using the Excel programme in Appendix B. Aeration was included in

the calculation. The calculated pH values were 9,2; 9,2 and 9,1 respectively for Runs A1, A2 and A3. These calculated pH values are much higher than the pH value of circa 5,50 on which the pHauxostat was operated. It indicates that the proton balance used in the calculation is incorrect because the history of the solution was not taken into account. Application of the equilibrium chemistry by this method will complicate the calculation and is not appropriate for the bio-pHauxostat.

ii) Equilibrium chemistry using solution and subsystem alkalinities

Applying the method from Loewenthal *et al.* (1991), the solution and subsystem alkalinities were calculated and the reactor solution characterised. Results are shown in Table 3.12 for Test Run A.

TABLE 3.12 - Calculated alkalinities for the substrate and reactor solutions: Test Run A (mol/*l*)

| Run | A1 | | A2 | | A3 | |
|---|---|---|---|---|---|---|
| Parameter | Substrate | Reactor | Substrate | Reactor | Substrate | Reactor |
| pH | 3,60 | 5,50 | 3,78 | 5,45 | 4,11 | 5,45 |
| Alk $H_2CO_3^*$ | $1,85 \times 10^{-8}$ | $1,43 \times 10^{-6}$ | $2,86 \times 10^{-8}$ | $1,29 \times 10^{-6}$ | $6,15 \times 10^{-8}$ | $1,32 \times 10^{-6}$ |
| Alk $H_2PO_4^-$ | $-9,51 \times 10^{-5}$ | $3,85 \times 10^{-5}$ | $-6,09 \times 10^{-5}$ | $4,11 \times 10^{-5}$ | $-2,46 \times 10^{-5}$ | $4,83 \times 10^{-5}$ |
| Alk $NH_3$ | -0,020775 | -0,006352 | -0,020775 | -0,007851 | -0,020775 | -0,007994 |
| Alk HAc | 0,012761 | ? | 0,018916 | ? | 0,036194 | ? |
| Alk $H_2O$ | -0,000299 | $-3,65 \times 10^{-6}$ | -0,000198 | $-4,15 \times 10^{-6}$ | $-9,54 \times 10^{-5}$ | $-4,29 \times 10^{-6}$ |
| $S_{ALK}$ | -0,008408 | -0,008408 | -0,002118 | -0,002118 | 0,015299 | 0,015299 |
| Calculated Alk HAc | -0,002092 | | 0,005695 | | 0,023248 | |
| HAc consumed (mg/*l*) | 10145 | | 9599 | | 8370 | |

Comparing the substrate subsystem alkalinities it can be seen that all the alkalinities increased with the increase in NaOH concentration except for Alk $NH_3$ that stayed constant. Considering that the $pK_N$ value for ammonia is circa 9,2 at 20°C (Weast 1974), it is expected that the slight increase in the pH in the acidic range, will have a negligible effect on the defined alkalinity, which is equal to the ammonia concentration. The negative alkalinities indicate that their equivalence points are at a

higher pH than the solution pH, which can also be defined as acidity (WRC 1986). The solution alkalinities increased with increase in pH, as would be expected.

The differences between the calculated and measured values of HAc consumed and the acetic acid concentrations ($S_A$), were calculated with the results shown in Table 3.13.

TABLE 3.13 - Difference in measured and calculated HAc values (mg/*l*): Test Run A

| Run | A1 | A2 | A3 |
|---|---|---|---|
| Measured HAc consumed | 9920 | 9660 | 8520 |
| Calculated HAc consumed | 10145 | 9599 | 8370 |
| Difference (mg/*l*) | +225 | -61 | -150 |
| Difference per measured % | +2,3 | -0,6 | -1,8 |
| Measured $S_A$ (Table 3.10) | 80 | 340 | 1480 |
| Calculated $S_A$ | -145 | 401 | 1630 |
| Difference (mg/*l*) | -225 | +61 | +150 |

Selection of reference species

The selection of the reference species was done purely on the basis of getting the most accurate results fitted to the measured values (Table 3.13), and not on a scientific biochemical basis. The HAc subsystem had the biggest influence, from the small difference given in the table, to a few hundred percent difference with acetate as the selected reference species. Nitrogen had the second biggest influence with differences in the order of 5 to 10% more for $NH_4^+$ versus $NH_3$ as reference species. Phosphorus had the smallest influence with the smallest difference indicated by species in-between $H_3PO_4$ and $PO_4^{3-}$.

Similarly to the above, the alkalinities and the differences in HAc consumed can be calculated for Test Run B. The results are shown in Table 3.14.

TABLE 3.14 - Difference in measured and calculated HAc values (mg/*l*): Test Run B

| Run | B1 | | B3 | |
|---|---|---|---|---|
| Parameter | Substrate | Reactor | Substrate | Reactor |
| PH | 3,88 | 5,51 | 3,88 | 5,53 |
| Alk $H_2CO_3^*$ | $3{,}36 \times 10^{-8}$ | $1{,}41 \times 10^{-6}$ | $3{,}36 \times 10^{-8}$ | $1{,}48 \times 10^{-6}$ |
| Alk $H_2PO_4^-$ | $-2{,}58 \times 10^{-5}$ | $1{,}49 \times 10^{-5}$ | $-2{,}58 \times 10^{-5}$ | $2{,}67 \times 10^{-5}$ |
| Alk $NH_3$ | -0,010423 | -0,003711 | -0,010423 | -0,004996 |
| Alk Hac | 0,011050 | ? | 0,011050 | ? |
| Alk $H_2O$ | -0,000152 | $-3{,}49 \times 10^{-6}$ | -0,000152 | $-3{,}35 \times 10^{-6}$ |
| $S_{ALK}$ | 0,000448 | 0,000448 | 0,000448 | 0,000448 |
| Calculated Alk Hac | 0,004147 | | 0,005419 | |
| Calculated HAc consumed | 4712 | | 4626 | |
| Measured HAc consumed | 4747 | | 4657 | |
| Difference (mg/l) | -35 | | -31 | |
| Difference per measured % | -0,7 | | -0,7 | |
| Measured $S_A$ (Table 3.11) | 253 | | 343 | |
| Calculated $S_A$ | 288 | | 374 | |
| Difference (mg/l) | +35 | | +31 | |

Calculated versus Measured values

The calculated and measured values of HAc consumption differs with less than three percent for Test Run A and less than one percent for Test run B, indicating good comparison. It is concluded that the calculation method works well for the pHauxostat and for the removal of chemical species from solution. The reactor solution is thereby characterised and all subsystem species are known.

**Species removed**

The results indicate that the yeast removes, on a net basis, specifically the species; HAc, $NH_3$ and $HPO_4^{2-}$ and/or $H_2PO_4^-$ from solution for growth. Comparing this to the oxidation-reduction reactions in Chapter II[1], it is noted that the acetate and ammonium species are the species indicated by the half reactions as taking part in the reaction. This is just the opposite to that calculated here. The small differences in the results and the sound basis of the method of calculation are taken to indicate correct calculation, although on an empirical basis.

**HAc concentration in the reactor**

The calculated and measured $S_A$ values differ more than the difference in HAc consumed, on a percentage basis (Tables 3.13 and 3.14). The difference for Run A1 seems especially high. The reason is that the acetic acid concentrations are relatively low, especially for Run A1, compared to the consumption values on which the calculation is based. Perfect test work and calculation will be required to improve these values. Run A1 does however show in general the biggest differences.

It was concluded that the HAc concentration in the reactor is determined by equilibrium chemistry and controlled by the substrate composition and set point pH. The set point pH and the substrate composition stayed the same for Runs B1 and B3, implying that $S_A$ should stay the same. Comparing the $S_A$ for Runs B1 and B3 (Table 3.11) it is seen that the concentration increased, which is somewhat unexpected. The N and P concentrations also increased. The only differences between the two runs are an increase in the HRT or decrease in growth rate and an expected decrease in the observed growth yield.

[1] *Vide* Chapter II p37

$S_A$ change with change in N and P concentrations at constant pH

The question is how does the $S_A$ change with a decrease in N and P consumption while the solution pH is kept constant. To answer this question the $S_A$ was calculated for an increase in N and P consumption, by using subsystem alkalinities. The results are shown in Table 3.15. The substrate composition was taken as: HAc = 5000 g/*l*, N = 146 mg/*l*, P = 51 mg/*l*, NaOH = 501 mg/*l* and reactor pH = 5,52 (Test Run B substrate).

TABLE 3.15 - Change in HAc concentration with increasing N and P consumption at constant pH

| Solution | N (mg/*l*) | P (mg/*l*) | Calculated $S_A$ (mg/*l*) | Measured $S_A$ (mg/*l*) |
|---|---|---|---|---|
| 1 | 120 | 40 | 620 | - |
| 2 | 100 | 35 | 522 | - |
| 3 (Run B3) | 70 | 27 | 375 | 343 |
| 4 (Run B1) | 52 | 16 | 287 | 253 |

From Table 3.15 it is clear that the $S_A$ decreases as the N and P concentrations decrease, to keep the pH constant. Solutions 3 and 4 are equivalent to Runs B3 and B1, respectively. The same tendency of a decrease in $S_A$ with a decrease in N and P concentrations was measured, confirming the calculated tendency. An explanation is that with the increase in the consumption of $NH_3$ or $H_2PO_4^-$ and/or $HPO_4^{2-}$ species (decrease in N and P concentration), an increased amount of hydrogen species is left behind in the solution. To keep the proton balance maintained at a constant pH, the HAc species is decreased. For the chemical-pHauxostat the base mainly neutralised the HAc to the set point pH. For the bio-pHauxostat the base neutralises the residual HAc concentration to the set point pH but also neutralises the net addition of protons from species removed. The base addition and the effect of other species removed will influence the residual $S_A$ in the reactor.

The increase in N and P concentrations may be due to a decrease in the percentage substrate removal or due to a change in the observed growth yield. A decrease in the observed growth yield

will result in a decrease in the consumption of nitrogen and phosphorus relative to HAc because nitrogen and phosphorus are consumed for cell synthesis, and cell synthesis decrease with a decrease in growth yield (Grady *et al.* 1999). To quantify this the ratios of N and P to HAc consumed and $Y_{obs}$ were calculated for Runs B1 and B3. The results are shown in Table 3.16.

TABLE 3.16 - Ratios of consumed N and P to HAc

| Run | B1 | B3 |
|---|---|---|
| HAc : N (measured) | 51 : 1 | 61:1 |
| HAc : P (measured) | 136 : 1 | 194:1 |
| HAc : N : P (measured) | 136 : 2,7 : 1 | 194 : 3,2 : 1 |
| $Y_{obs}$ (X/HAc)* | 0,272 | 0,258 |
| Calculated $S_A$** | 287 | 375 |
| HAc : N : P (calculated) | 135 : 2,7 : 1 | 193 : 3,2 : 1 |

* $Y_{obs}$ calculated as biomass produced per acetic acid consumed

** Refer Table 3.15

The ratios indicate a decrease in N and P consumption relative to HAc consumption with a decrease in the observed growth yield, which decreased with a decrease in the SRT (HRT), as expected. It follows that the difference in the $S_A$ between Runs B1 and B3 is due to the action of equilibrium chemistry induced by the decrease in the observed growth yield. This indicates that the concentrations of the nutrients forming part of the weak acid/base subsystems change in a complex manner, to satisfy both the nutrients required for growth and the equilibrium chemistry to keep the pH constant.

**Change in buffer intensity**

The buffer intensity of a solution is represented by the sum of the buffer intensities of all the weak acid/base subsystems in solution (WRC 1992). Changes in the weak acid/base subsystem species will change the respective buffer intensities. The results in Table 3.10 indicate that the total

subsystem species for the acetate, ammonium and phosphate subsystems decreased with growth, implicating that the respective buffer intensities also decreased. The buffer intensities for Run A2 were calculated (Stumm & Morgan 1981) and are demonstrated in Figs. 3.2, 3.3 and 3.4, representing respectively the buffer intensities for the acetic acid, nitrogen and phosphorus subsystems, each together with the buffer intensity of water. The substrate pH, $pH_s$, and the reactor solution pH, $pH_r$, are indicated in the figures. The areas under the buffer intensity curves between the solution pH and the equivalence point pH represent the respective alkalinities. **The equivalence points are indicated with pointers in the figures**. The buffer intensities for the carbonate subsystem are for both the feed and the reactor solutions negligible and are not shown.

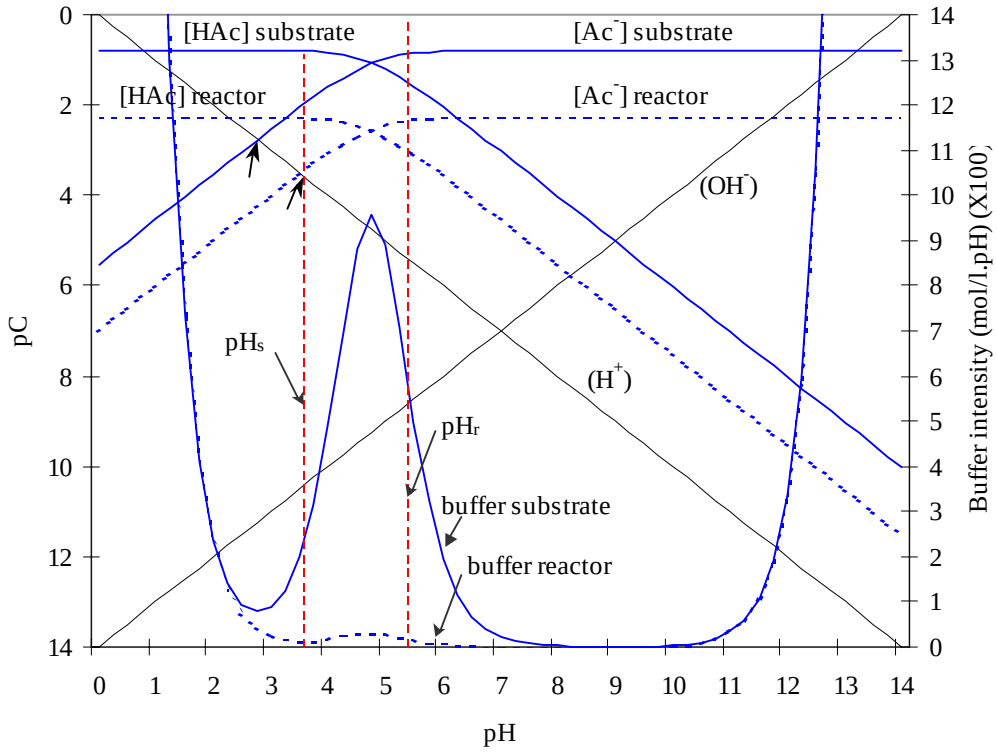


FIG. 3.2 - The change in buffer intensity for the acetate subsystem

From these results and the discussion in Chapter II, it is clear that the effect of the base added to the substrate will relate to the changed buffer solution (in the reactor) and not to the feed solution. This is taken into account by the alkalinity in the reactor.

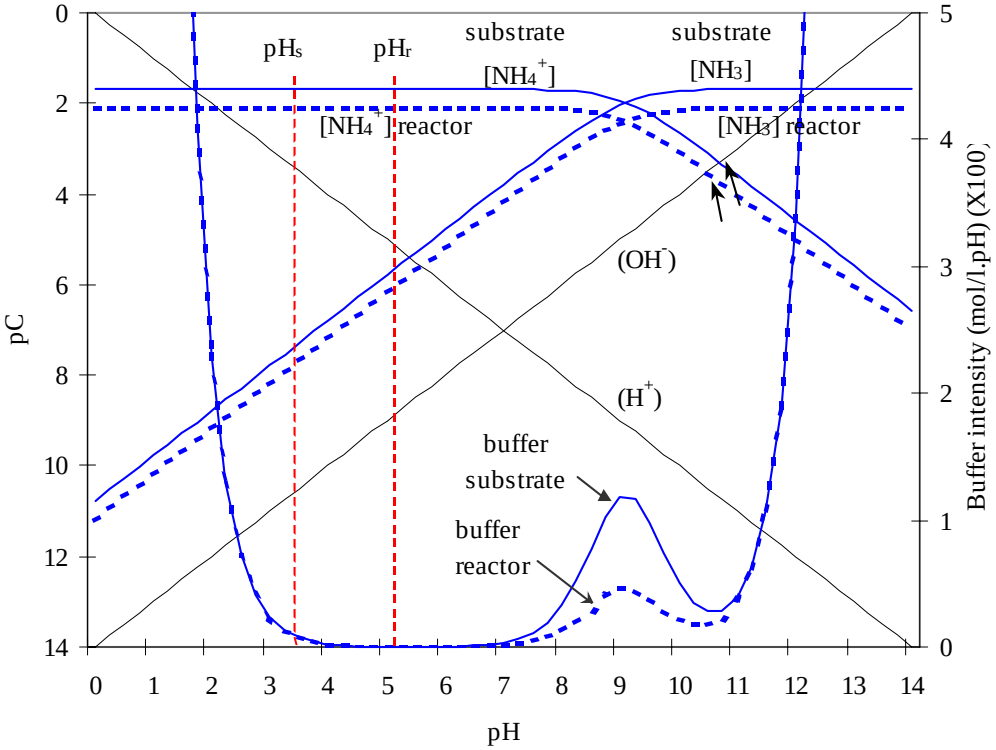


FIG. 3.3 - The change in buffer intensity for the nitrogen subsystem

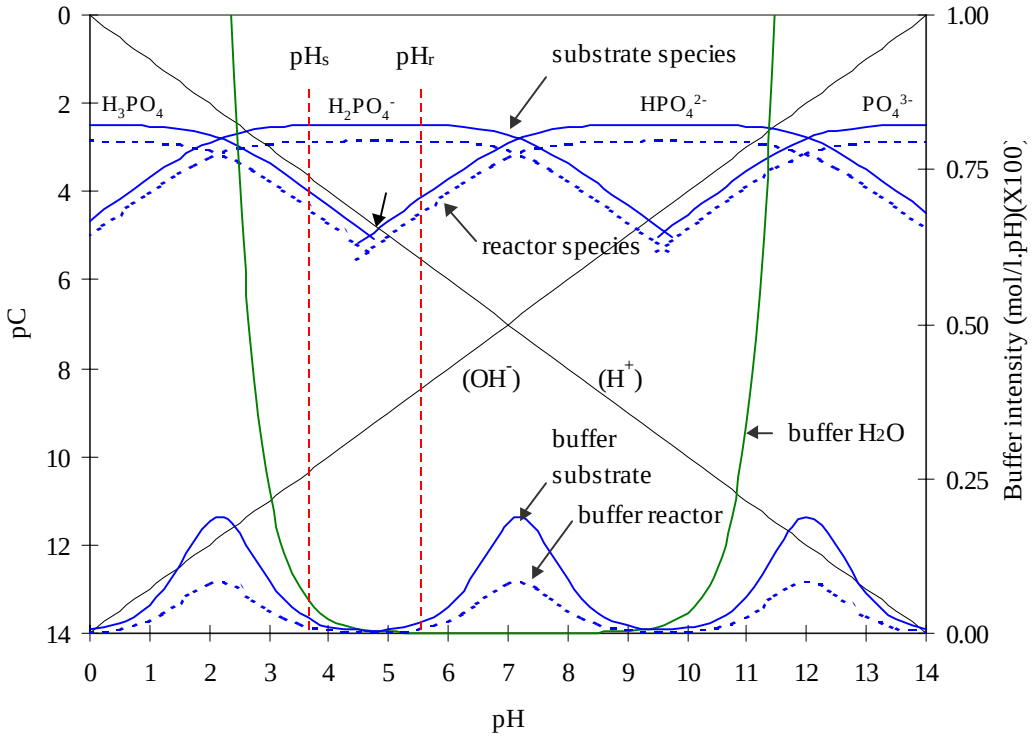


FIG. 3.4 - The change in buffer intensity for the phosphorus subsystem

Accuracy of control

An aspect related to the buffer intensity is the accuracy of control. The sensitivity of the pH measurement will be decreased with the measurement point in a pH range of high buffer intensity, resulting in a decreased control sensitivity and accuracy. Pretorius (1995) demonstrated this by decreasing the buffer intensity by stripping carbon dioxide in a sideline of an anaerobic process and thereby increasing the control sensitivity.

**Calculating the alkalinity difference**

The developed Eq. 17 utilises the difference in alkalinity in the calculation, but the equivalence point changes as the subsystem species are removed, demonstrated in Chapter II[1] and Figs. 3.2 to 3.4, and thereby changing the reference point for alkalinity measurement. A concern might be that the change in the reference point might influence the difference in alkalinity, with an alternative of using a fixed pH as reference point. The change in the reference point is however taken into account by a change in the solution pH. This can be demonstrated by considering a solution with only HAc added. The solution alkalinity is the acetic acid alkalinity, represented by:

$$\begin{aligned} \text{Solution alkalinity} &= \text{Alk HAc} + \text{Alk } H_2O \\ &= [Ac^-] + [OH^-] - [H^+] \\ &= 0 \text{ mol}/l \end{aligned}$$

The alkalinity is zero because it is an equivalent solution and by removing HAc species from solution the alkalinity must stay zero, because it stayed an equivalent solution. The equivalence point however moved, with an associated increase in the pH (Fig. 3.2). The alkalinity seems to increase, because the pH increased, which is contradictory. A similar calculation demonstrating these changes was done, with results shown in Table 3.17. From the values in the table it can be seen that the alkalinity of the water subsystem increased (due to the pH increase) but this increase was counteracted by a decrease in the alkalinity of the acetate subsystem. It demonstrates that although the equivalence point changed, the change was taken into account by a change in the solution pH and subsystem alkalinities, to result in the correct solution alkalinity. This makes the equivalence point the correct reference point, and is the direct use of the difference in alkalinity correct.

[1] *Vide* Chapter II Fig. 2.4 p33

TABLE 3.17 - The change in subsystem alkalinities for an equivalent solution

| Parameter | Solution 1 | Solution 2 |
|---|---|---|
| HAc (mg/*l*) | 5000 | 1000 |
| PH | 2,916 | 3,271 |
| Alk HAc (mol/*l*) | 0,0012 | 0,0005 |
| Alk $H_2O$ (mol/*l*) | -0,0012 | -0,0005 |
| $S_{ALK}$ (mol/*l*) | 0 | 0 |

A potential error is the incorrect measurement of the solution pH. This may, according to Linder, Torrington & Williams (1984), be due to a systematic pH measurement error, caused by incorrect calibration and by differences in the ionic strength between the calibration solution and the sample, besides human errors. This type of error however falls outside the scope of this study.

**Theory application**

The test results (Tables 3.10 and 3.11) were used in Eqs. 16 and 19 to calculate respectively the measured and theoretical alkalinity yields. The reference species for the subsystem alkalinities were selected so that a change in the solution alkalinity between the substrate and reactor solutions will result. The selected reference species were $Ac^-$, $NH_4^+$, $H_3PO_4$ and $CO_3^{2-}$. Any species may be selected except the species removed or added (used in the previous calculation) (Loewenthal *et al.* 1991). The subsystem and solution alkalinities are presented in Tables 3.18 and 3.19, for Test Runs A and B, respectively.

The calculated yields are given in Tables 3.20 and 3.21. $Y_{Alk}$(m) represents the measured yield, calculated by using Eq. 16, and $Y_{Alk}$(t), the calculated theoretical yield, calculated by using Eq. 19. The measured and theoretical yield values compares well, with differences within 1%, except for Run B3 that is 2%.

TABLE 3.18 - Subsystem and solution alkalinities (mol/*l*) for calculating $Y_{ALK}$ : Test Run A

| Run | A1 | | A2 | | A3 | |
|---|---|---|---|---|---|---|
| Parameter | Substrate | Reactor | Substrate | Reactor | Substrate | Reactor |
| pH | 3,60 | 5,50 | 3,78 | 5,45 | 4,11 | 5,45 |
| Alk $CO_3^{2-}$ | $-1{,}71 \times 10^{-5}$ | $-1{,}85 \times 10^{-5}$ | $-1{,}71 \times 10^{-5}$ | $-1{,}84 \times 10^{-5}$ | $-1{,}69 \times 10^{-5}$ | $-1{,}82 \times 10^{-5}$ |
| Alk $H_3PO_4$ | 0,003166 | 0,001330 | 0,003200 | 0,001526 | 0,003236 | 0,001630 |
| Alk $NH_4^+$ | $6{,}59 \times 10^{-8}$ | $1{,}55 \times 10^{-6}$ | $1{,}02 \times 10^{-7}$ | $1{,}74 \times 10^{-6}$ | $2{,}29 \times 10^{-7}$ | $1{,}89 \times 10^{-6}$ |
| Alk $Ac^-$ | -0,153762 | -0,000180 | -0,147607 | -0,000835 | -0,130329 | -0,003537 |
| Alk $H_2O$ | -0,000299 | $-3{,}65 \times 10^{-6}$ | -0,000198 | $-4.15 \times 10^{-6}$ | $-9{,}54 \times 10^{-5}$ | $-4{,}29 \times 10^{-6}$ |
| $S_{ALK}$ | -0,150912 | 0,001129 | -0,144622 | 0,000671 | -0,127205 | -0,001927 |
| $S_{ALK}$-$S_{ALK0}$ | 0,152041 | | 0,145293 | | 0,125278 | |

TABLE 3.19 - Subsystem and solution alkalinities (mol/*l*) for calculating $Y_{ALK}$: Test Run B

| Run | B1 | | B3 | |
|---|---|---|---|---|
| Parameter | Substrate | Reactor | Substrate | Reactor |
| pH | 3,88 | 5,51 | 3,88 | 5,53 |
| Alk $CO_3^{2-}$ | $-1{,}66 \times 10^{-5}$ | $-1{,}80 \times 10^{-5}$ | $1{,}66 \times 10^{-5}$ | $-1{,}81 \times 10^{-5}$ |
| Alk $H_3PO_4$ | 0,001621 | 0,000531 | 0,001621 | 0,000898 |
| Alk $NH_4^+$ | $6{,}59 \times 10^{-8}$ | $9{,}82 \times 10^{-7}$ | $6{,}59 \times 10^{-8}$ | $1{,}38 \times 10^{-6}$ |
| Alk $Ac^-$ | -0,072212 | -0,000569 | -0,072212 | -0,000738 |
| Alk $H_2O$ | -0,000153 | $-3{,}49 \times 10^{-6}$ | -0,000153 | $-3{,}35 \times 10^{-6}$ |
| $S_{ALK}$ | -0,070760 | -0,000058 | -0,070760 | 0,000140 |
| $S_{ALK} - S_{ALK0}$ | 0,070702 | | 0,070900 | |

TABLE 3.20 - Alkalinity Yields: Test Run A

| Run | A1 | A2 | A3 |
|---|---|---|---|
| X (g SS/*l*) | 2,00 | 2,66 | 2,30 |
| $Y_{obs}$ (SS/HAc) | 0,202 | 0,275 | 0,270 |
| $S_{ALK} - S_{ALK0}$ | 0,152041 | 0,145293 | 0,125278 |
| $Y_{ALK}$ (m) (mol/mol) | 0,92 | 0,90 | 0,88 |
| $f_s$ ($Y_{obs}$:COD/COD) | 0,205 | 0,280 | 0,275 |
| $Y_{ALK}$ (t) (mol/mol) | 0,92 | 0,89 | 0,89 |
| Percentage difference | 0 | -1 | +1 |

TABLE 3.21 - Alkalinity Yields: Test Run B

| Run | B1 | B3 |
|---|---|---|
| X (g SS/*l*) | 1,29 | 1,20 |
| $Y_{obs}$ (SS/HAc) | 0,272 | 0,258 |
| $S_{ALK} - S_{ALK0}$ | 0,070702 | 0,070900 |
| $Y_{ALK}$ (m) (mol/mol) | 0,89 | 0,91 |
| $f_s$ ($Y_{obs}$ : COD/COD) | 0,277 | 0,262 |
| $Y_{ALK}$ (t) (mol/mol) | 0,89 | 0,89 |
| Percentage difference | 0 | -2 |

The difference in alkalinity yield is the biggest for Run B3, with a difference of 2%. It is still within an acceptable range considering that the accuracy of equilibrium constants for weak acids and bases may vary by $\pm$ 10 percent (Sawyer *et al.* 1994). It confirms the proposed theory but also indicate a possible deviation for Run B3. The main difference between Run B3 and the other runs

analysed is that the GLN was oxygen for Run B3 versus HAc for the other runs. A possible explanation is that the equation assumed for biomass might be different in the case of oxygen limitation (Blanch & Clark 1997). Rice and Hempfling (1978) also reported that oxygen limitation substantially lowers the rate of maintenance respiration as compared to continuous cultures limited by succinate and tested in a pHauxostat.

Predicted change by theory

The difference in the solution alkalinities between the substrate and reactor solutions decreased from Run B1 to Run B3. It represents the right side of Eq. 16 and implicates a decrease of the left side, if the equation is correct. With $Y_{ALK}$ increasing slightly and $Y_{obs}$ decreasing slightly, with the decrease in the growth rate, it means that the biomass concentration needs to decrease to satisfy the equation. In Table 3.11 it can be seen that this is precisely what happened in the laboratory test work. It demonstrates the correct prediction by the theory.

These results confirm that alkalinity and the derived Eq. 16, together with the defined alkalinity yield coefficient, can be used to define the pHauxostat process. The theoretical alkalinity yield coefficient is also successful in predicting the value of the expected yield coefficient.

**Contributions of subsystems to $Y_{ALK}$**

Considering that the half reactions and the theoretical alkalinity yield do not incorporate the change in pH between the substrate and reactor solutions, the phosphorus as a nutrient and the carbonate as an open system, then the differences are unexpectedly small. All these aspects influence the solution alkalinity and thus the alkalinity yield. The answer is seen in comparing the different subsystem alkalinities and calculating the change in each subsystem alkalinity. The change in the subsystem alkalinities between the substrate and reactor solutions for Run A2, are shown in Table 3.22.

From the table it is clear that the change in the solution alkalinity is mainly due to Alk $Ac^-$ which represents 98,7% of the absolute difference in alkalinity. Alk $H_3PO_4$ represents 1,1%, as the second biggest. To compare the measured and theoretical yields at par, $Y_{ALK}$ (m) was recalculated by only taking the change in Alk $Ac^-$ into account, giving values of 0,93; 0,91 and 0,89 for Runs A1, A2 and A3 respectively. These values are very similar as previously calculated (Table 3.20) with

approximately the same percentage differences. This explains the reason why the considered half reactions give a close approximation of the alkalinity yield in these cases. It also indicates that

TABLE 3.22 - Change in alkalinities for Run A2 : Absolute differences

| Alkalinity | Substrate | Reactor | Absolute difference |
|---|---|---|---|
| Alk $CO_3^{2-}$ | $-1{,}713 \times 10^{-5}$ | $-1{,}839 \times 10^{-5}$ | $1{,}26 \times 10^{-6}$ |
| Alk $H_3PO_4$ | 0,0031999 | 0,0015263 | 0,001674 |
| Alk $NH_4^+$ | $1{,}018 \times 10^{-7}$ | $1{,}736 \times 10^{-6}$ | $1{,}63 \times 10^{-6}$ |
| Alk $Ac^-$ | -0,1476074 | -0,0008348 | 0,146773 |
| Alk $H_2O$ | -0,0001976 | $-4{,}155 \times 10^{-6}$ | 0,000193 |
| Absolute difference, $S_{ALK}$ | - | - | 0,148643 |

should a subsystem that is not represented in the half reactions contribute significantly to the change in alkalinity, then the theoretical yield will not give accurate predictions.

## 2.4 Conclusions

The pHauxostat performed well with a stable operation by self-control via the feed system. The feed rate and therefore the growth rate could be manipulated for a Category A pHauxostat by the amount of base added to the substrate, which also changed the growth limiting nutrient concentration. The test work also demonstrated a Category B pHauxostat indicating Self-regulation similar to a Category A pHauxostat but with a changed control sequence. The growth rate for a Category B pHauxostat is controlled by the supply rate of the GLN.

The proposed equilibrium chemistry method for characterising the pHauxostat, the proposed theory and the developed equations, were demonstrated to be the same for Category A and B pHauxostats. The test work also demonstrated that the pHauxostat technique can be operated with a gaseous nutrient as the GLN.

## 3. CONCLUSIONS

- The pH, which is a function of the weak acid/base subsystems and added strong acid or base, is the controlled Output variable for the pHauxostat with the feed the manipulated Input variable.

- The reactor solution can be characterised by using solution and subsystem alkalinities together with equilibrium chemistry.

- The feed rate for the pHauxostat are determined and controlled by the difference in the solution alkalinities between the substrate and reactor solutions and the alkalinity generation rate, expressed by:

$$F = r_{ALK} V / (S_{ALK} - S_{ALK0}) \quad \text{(Eq. 24)}$$

with $$r_{ALK} = \mu i_s X_{COD} Y_{ALK} / Y_{obs} \quad \text{(Eq. 25)}$$

- pHauxostats may be categorised in Category A and B pHauxostats with S = f(pH) for Category A and S ≠ f(pH) for Category B, and further subdivided on the basis whether the GLN is part of the manipulated feed or not.

- The GLN concentration and thus the growth rate for Category A pHauxostats are controlled by the pH set point and substrate composition, while the biomass concentration is determined by the difference in the solution alkalinities between the substrate and the reactor.

- The GLN concentration for Category B pHauxostats is not controlled by, but is a result of the control method that controls the feed rate and thereby the growth rate. The biomass concentration is determined by the load rate of the GLN.

- The change in solution alkalinity may be defined by a theoretical alkalinity yield, based on oxidation-reduction half reactions when the change is mainly due to the change in the substrate, and is expressed as:

$$Y_{ALK} = x + Y_{obs} y \quad \text{(Eq. 20)}$$

or $Y_{ALK} = (Y_{TALK} + xb\tau) / (1 + b\tau)$ (Eq. 22)

- The pHauxostat can be modelled by the change in solution alkalinity, which is represented by an alkalinity yield coefficient. In combination with growth kinetics, the pHauxostat is modelled by the equations:

$$i_s X_{COD} Y_{ALK} / Y_{obs} = S_{ALK} - S_{ALK0} \quad \text{(Eq. 16)}$$

or $i_s X_{COD} Y_{ALK} (1 + b\tau) / Y = S_{ALK} - S_{ALK0}$ (Eq. 17)

# CHAPTER IV - APPLICATION AND DEMONSTRATION

*In the first part of this chapter the developed theory is applied/tested on actual petrochemical effluent, treated in a demonstration pHauxostat plant. In the second part plots are used to demonstrate and visualise the correlation between the different parameters for pHauxostats in general.*

## 1. APPLICATION

### 1.1 Introduction

The lay-out used to explore and explain the principles of the pHauxostat in the previous chapters, had a chemostat lay-out. This implies that the hydraulic residence time and the biomass retention time or solids retention time are the same. To decouple the HRT and the SRT for a more flexible and controllable continuous culture process, a lay-out with cell recycle is generally applied in full scale continuous culture processes (Grady *et al.* 1999). Fraleigh *et al.* (1989) investigated auxostats and concluded that the commercial application of auxostats will probably require a configuration with multistage reactors or with recycle. The opportunities for full scale application of the pHauxostat would rather be with a lay-out with cell recycle, besides the possible fed-batch application.

No published studies or any of the literature collected, referred to, or applied a lay-out with cell recycle. Strictly speaking an exception is the test work on start-up of anaerobic digestion. In this lay-out the biomass is kept in the reactor due to settling (Upflow Anaerobic Sludge Blanket) or due to fixed growth (Fixed Growth Anaerobic Process) (Speece 1996).

To test the commerciality of the pHauxostat technique and apply the developed theory on an actual effluent as substrate, a pHauxostat with cell recycle was applied in treating a petrochemical effluent in a demonstration pHauxostat plant.

## 1.2 Modelling

The equation development in Chapter II was for a pHauxostat with a chemostat lay-out, and the derived Eqs. 16 and 17 need to be adapted to a lay-out with cell recycle. Figure 4.1 demonstrates a reactor with a biomass separator (cell recycle). This lay-out is different to the lay-out of a chemostat, in that the biomass separator decouple the HRT and the SRT and a biomass wastage stream, $F_w$, is added.

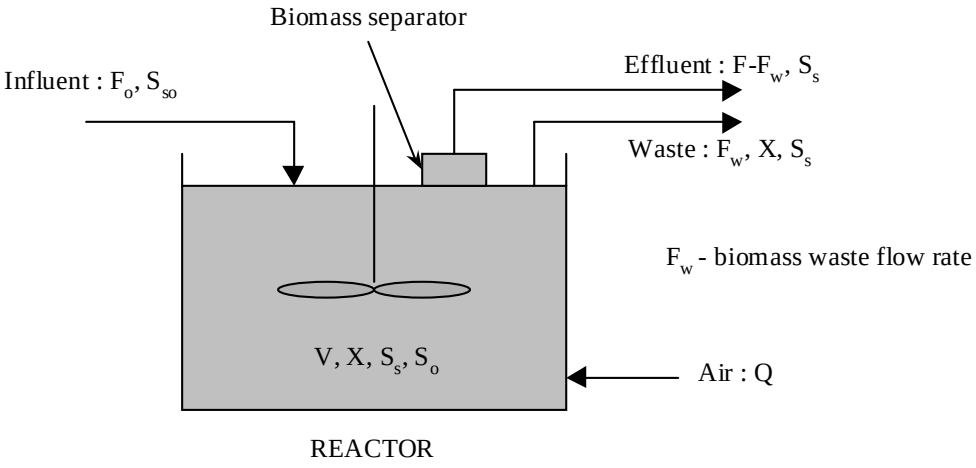


FIG. 4.1 - CSTR with biomass separator

In analogy with Chapter II a mass balance on alkalinity is done:

$$V\,d\text{alk}/dt = F_oS_{ALK0} - F_wS_{ALK} - (F - F_w)\,S_{ALK} + \text{alkalinity generation rate}$$
$$= F_oS_{ALK0} - FS_{ALK} + \text{alkalinity generation rate}$$
$$\therefore\ d\text{alk}/dt = (F_o/V)\,S_{ALK0} - (F/V)\,S_{ALK} + \text{alkalinity generation rate per volume}$$

The result is the same as for Eq. 13 because $S_{ALK}$ for the effluent and waste streams is the same. The rate for alkalinity production per volume, $r_{ALK}$, and $r_s$ are defined by:

$$r_{ALK} = i_sY_{ALK}\,r_s \qquad (14)$$
$$r_s = -(F/V)\,(S_{so} - S_s) \qquad (A7)$$

with $X_B$ given by Eq. 8 (for cell recycle) and assuming negligible debris results in $X_B$ being equal to X and reveals Eqs. 26 and 27:

$$X_B = (\theta_c/\tau)\ [Y\ (S_{so} - S_s)]\ /\ (1 + b\theta_c) \quad (8)$$

$$r_s = -\ (F/V)\ (X/Y)\ (1 + b\theta_c)\ (\tau/\theta_c) \quad (26)$$

$$r_{ALK} = (F/V)\ (X_{COD}\ i_s\ Y_{ALK} / Y)\ (1 + b\theta_c)\ (\tau/\theta_c) \quad (27)$$

in combination with the mass balance on alkalinity and for steady state:

$$i_s\ X_{COD}\ Y_{ALK}\ (1 + b\theta_c)\ (\tau/\theta_c) / Y = S_{ALK} - S_{ALK0} \quad (28)$$

Eq. 28 is the equivalent to Eq. 17 but for a pHauxostat with cell recycle. Completing a mass balance on biomass concentration, an equation for $Y_{obs}$ may be derived in terms of Y, the true growth yield (Grady *et al.* 1999 : 155):

$$Y_{obs} = (1 + f_D b\theta_c)\ Y\ /\ (1 + b\theta_c) \quad \text{(Grady } et\ al\text{. 1999, Eq. 5.28)}$$

with $f_D$ the fraction of active biomass contributing to biomass debris, but with the assumption that debris is negligible and therefore $f_D = 0$, an equation similar to Eq. 7 is derived:

$$Y_{obs} = Y / (1 + b\theta_c) \quad (29)$$

Substituting Y in Eq. 28 with Eq. 29, giving Eq. 30 reveals the equivalent of Eq. 16:

$$i_s\ X_{COD}\ Y_{ALK}\ (\tau/\theta_c) / Y_{obs} = S_{ALK} - S_{ALK0} \quad (30)$$

Assuming that alkalinity generation is adequately represented by the theoretical half reactions, an equation similar to Eq. 23 is derived by combination of Eqs. 21, 28 and 29 giving:

$$i_s\ X_{COD}\ (\tau/\theta_c)\ (Y_{TALK} + xb\theta_c) / Y = S_{ALK} - S_{ALK0} \quad (31)$$

The equations for a pHauxostat with cell recycle or biomass separator are similar to that for a chemostat lay-out, but decouple the hydraulic and sludge retention times.

## 1.3 Control Methodology

The lay-out has the general benefit of independently controlling the SRT and thus the growth rate. This implies that it can not be a Category A pHauxostat, with S = f(pH), because S, the GLN concentration, is controlled by external control of the SRT. It must be a Category B pHauxostat, with $S \neq f(pH)$.

Assuming that all the nutrients are in excess relative to a carbon and energy source, for example HAc, then the substrate composition and selected pH set point will determine the HAc concentration in the reactor (Chapters II and III). But in a cell recycle lay-out, the growth rate and the GLN concentration is determined by the selected SRT. Should the HAc concentration be lower than the required GLN concentration for the selected SRT, then the biomass will be wasted faster than replenished and the process will fail. In contrast, should the HAc concentration be higher than the required GLN concentration, then growth will be faster than wasted and the biomass will increase. The increased biomass concentration will increase the feed flow rate with an ever-increasing biomass, until something fails or limits further increase, for example the feed-pump capacity. This will result in the pH increasing above the pH set point with loss of control. Alternatively another nutrient may get limiting, for example oxygen, which is added independently from the manipulated feed. The increase in biomass concentration will in this case increase the oxygen utilisation rate until it matches the oxygen supply rate, thereby resulting in oxygen limitation. The DO concentration required by the controlled growth rate will result, similar to the Category B pHauxostat with oxygen limitation explained in Chapter II, but with the growth rate controlled independently from the HRT. The HRT will be determined by the difference in the alkalinity between the substrate and the reactor solutions and by the alkalinity yield together with the alkalinity generation rate, as explained previously. It follows that any nutrient may be the GLN which is not influenced by the pH, as explained for a Category B pHauxostat.

### Control of the HRT

The HRT may be manipulated by the control of the difference in the solution alkalinities. This can be done as was done in the chemo-pHauxostat, by a controlled and independent addition of alkalinity or acidity to the reactor (or substrate). The feed rate may thereby be increased or decreased and the HRT be changed. This is possible because the GLN concentration is not influenced by the change in feed rate, as long as the non-GLN concentrations are higher than that

required for growth limitation. It will however increase or decrease the residual substrate concentration in the effluent.

An alternative would be to exchange the manipulated feed rate, for a manipulated substrate feed concentration, and independently control the feed flow rate and the HRT. This will result in a controlled treated effluent quality, but will necessitate a dilution water stream. This is only possible if the required substrate concentration is less than the undiluted substrate concentration at the intended HRT. This configuration was applied in the demonstration plant, using an acidic petrochemical effluent as substrate and a fungus as culture.

## 1.4 Oxygen uptake and transfer

To analyse and interpret test results with oxygen as the GLN, it will be necessary to consider the oxygen uptake (RO) and transfer rates. The uptake rate can be calculated by doing a mass balance on COD over the process, and the transfer rate can be calculated with the oxygen supply rate known (Grady *et al*. 1999). The oxygen uptake is dependent on the amount of biomass in the reactor and the SRT, with an increase in RO with an increase in biomass and in SRT (Grady & Lim 1980).

Considering oxygen transfer; the aspects influencing oxygen transfer includes the air supply rate, aeration equipment, the difference in oxygen concentration as the driving force, and hydrodynamics, which influences $K_La$, the mass transfer coefficient and the gas-liquid interfacial area (Bailey & Ollis 1986). The value of $K_La$ is influenced by the solution viscosity, with the solution viscosity influenced by the biomass concentration, especially in the case of filamentous microorganisms (Brierly & Steel 1959). The biomass concentration will therefore influence oxygen transfer.

## 1.5 Experimental Methods

### Substrate

An acidic organic effluent stream from a petrochemical industry containing ca. 1,1 to 1,2% $C_2$-$C_5$ monocarboxylic acids was used as substrate (Table 4.1). Although the total concentration of the monocarboxylic acids varied, the ratios of the individual acids remained constant (Augustyn 1995).

Substrate was taken from an online effluent stream, keeping a 100 $m^3$ storage tank filled-up and acting as an equalisation tank. Nitrogen in the form of ammonia gas, phosphoric acid and potassium sulphate (all industrial grade) were added as nutrients (Table 4.2). Sufficient micronutrients were present in the effluent stream and the added macronutrients for the process to function and no other growth factors were added.

TABLE 4.1 - Typical substrate composition (Augustyn 1995)

| Component | Concentration mg/*l* |
|---|---|
| acetic acid | 7650 |
| propionic acid | 2193 |
| i-butyric acid | 324 |
| n-butyric acid | 725 |
| i-valeric acid | 252 |
| n-valeric acid | 213 |
| methanol | 132 |
| ethanol | 32 |

TABLE 4.2 - Nutrients added (industrial grade)

| Chemical | Addition per 12 g of total acids |
|---|---|
| $NH_3$ (gas) | ca 0,50 g |
| $H_3PO_4$ - P | ca 0,120 g |
| $K_2SO_4$ | ca 0,23 g |

**Demonstration plant lay-out**

The plant lay-out is shown in Fig. 4.2. The pH-controller controlled the substrate-pump and the nutrient-pump, switching them simultaneously on and off with manual flow rate setting. The substrate was pumped to a mixing and dilution tank and the nutrients directly to the reactor. The diluted substrate was pumped via a manually controlled feed-pump to the reactor. Ammonia gas was controlled manually and added to the diluted feed stream with measurement in a rotameter. Potable water was used as dilution water in a make-up fashion. No alkali was added.

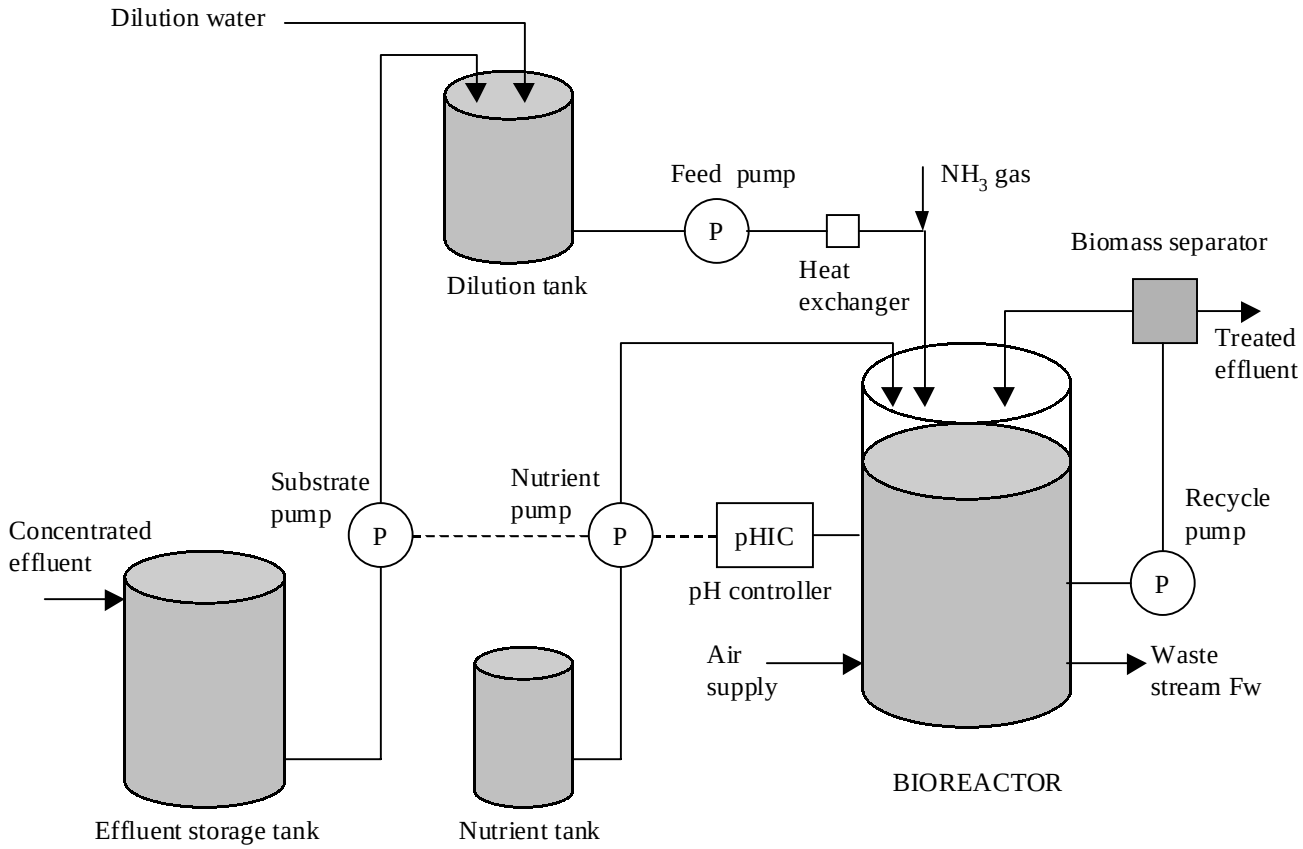


FIG. 4.2 - Demonstration plant lay-out

The reactor had a working volume of ca 6000 *l* and was operated as an open system (non-aseptic). A screen type biomass separator was used (Kühn & Pretorius 1988, Kühn & Pretorius 1989). The reactor temperature was controlled by heating the feed stream in a heat exchanger.

**Culture**

Three fungus species; *Geotrichum candidum*, *G. fragrans* and *G. ingens*, were naturally selected and simultaneously present, with morphology and growth kinetics very similar (van der Westhuizen 1993 & Augustyn 1995). Selection pressure was used to select and maintain the fungus species with minimum contamination (Pretorius 1987).

**Test runs, monitoring and analyses**

The demonstration plant was operated continuously over a ten-month period with minimal shutdowns. Four test runs were selected from the data for different SRT's and air supply rates (Table 4.3). The plant was monitored hourly. The hourly readings were collated to give daily results. Two composite samples were taken daily from the reactor feed and treated effluent and analysed for dissolved COD, SCFA, suspended solids (only treated effluent) and routinely for ammonia and phosphate. Other macronutrients were intermittently analysed for; Mg, K and Ca. The reactor contents were sampled twice daily for suspended solid determination. The temperature, pH and DO were continuously monitored. All analyses were done as per Standard Methods (1980) except where otherwise stated. The SCFA was determined by gas chromatography and expressed as HAc. Microscopic observation was daily done by phase contrast microscope for physiological observations of growth. Microbial population and contamination were monitored using standard plate count methods (Gerhardt *et al.* 1981; Augustyn 1995).

## 1.6 Results and Explanation

The results are given in Tables 4.3 and 4.4 as average values with standard deviations. The number of data points for each average value is given. Each data point represents a successful 24 hour operational period with its associated successful analyses.

TABLE 4.3 - Demonstration plant test results; SRT, COD and SCFA

| Run | V/V* | Data points | SRT (h) | | Feed COD (g/*l*) | | Feed SCFA (g/*l*) | | Effluent COD (g/l) | | Effluent SCFA (g/*l*) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | avg | st. dev. | avg | st. dev. | avg | st. dev. | avg | st. dev. | avg | st. dev. |
| 1 | 0,64 | 12 | 11,3 | 0,8 | 3,35 | 0,23 | 2,61 | 0,20 | 0,54 | 0,08 | 0,23 | 0,09 |
| 2 | 0,85 | 16 | 17,9 | 0,8 | 3,96 | 0,49 | 2,72 | 0,46 | 0,79 | 0,17 | 0,47 | 0,10 |
| 3 | 1,35 | 20 | 12,3 | 1,2 | 4,34 | 0,65 | 3,40 | 0,49 | 0,62 | 0,18 | 0,18 | 0,09 |
| 4 | 1,30 | 30 | 15,3 | 0,7 | 4,03 | 0,35 | 3,04 | 0,41 | 0,61 | 0,16 | 0,20 | 0,17 |

* V/V: Volume air per volume reactor contents per minute.

TABLE 4.4 - Demonstration plant test results; X, temperature, pH, N, P and DO

| Run | X (g/*l*) | | Temp. °C | pH | | Nitrogen (mg/*l*) | | Phosphorus (mg/*l*) | | DO Reactor (mg/*l*) |
|---|---|---|---|---|---|---|---|---|---|---|
| | avg. | st. dev. | | Feed | Reactor | Feed | Effl | Feed | Effl. | |
| 1 | 3,55 | 0,33 | 31,7 | 4,13 | 4,84 | 105 | 2 | 24 | 2 | 0,7 |
| 2 | 4,27 | 0,33 | 29,3 | 4,06 | 4,91 | 97 | 3 | 21 | 6 | 0,6 |
| 3 | 4,62 | 0,45 | 30,0 | 4,14 | 5,05 | 148 | 8 | 31 | 3 | 0,5 |
| 4 | 4,63 | 0,66 | 29,9 | 4,16 | 5,00 | 128 | 7 | 25 | 5 | 0,6 |

**General plant operation**

The accurate operational control and measurement achieved in the laboratory test work could not be achieved in the demonstration plant, mainly due to practical and operator associated reasons. The pHauxostat technique proved however to be very reliable even with operator errors. Its self-regulating nature of operation resulted in near instant indication of any operational problems. Any process variables that influences growth are directly indicated by a change in the undiluted substrate feed rate or substrate feed concentration and/or change in the DO concentration. The quick response in the output variables is a benefit of the pHauxostat technique, plus the self-regulated adaptation to any change in the input variables.

**The GLN**

The results indicate that the nutrients were in excess except for oxygen that had a low concentration and stayed low even with a doubling in the air supply, indicating oxygen limitation. The DO concentrations do not correlate with the SRT's, as may be expected for the GLN. Reasons might be that the DO concentration does not change significantly within the range of SRT's tested, and that measurement was not sensitive enough. Both these reasons are expected to have contributed to the near static DO readings.

**Equilibrium chemistry and subsystem alkalinities**

The application of equilibrium chemistry and calculations by subsystem alkalinities were demonstrated in the previous chapter using defined and laboratory prepared substrate, the question is whether the same results are possible using actual effluent as in this case. Using the results in Tables 4.3 and 4.4, the subsystem alkalinities were calculated and given in Table 4.5. The consumed HAc and the $S_A$ were calculated and compared to the measured results, with differences shown in Table 4.6.

The differences between the measured and calculated values of HAc consumed are small with values of 2% and less, except for Run 2 that is over 10%. The reason for this higher value is not clear, it is also a run with an increased effluent SCFA concentration that might have some connection to the bigger difference. The differences, even for Run 2 however, indicate a good comparison between the calculated and the actual results considering acceptable differences in equilibrium chemistry as being less than 15% (Snoeyink & Jenkins 1980). The relative low feed concentrations resulted in differences in $S_A$ that are less than that of the laboratory test work.

TABLE 4.5 - Subsystem and solution alkalinities (mol/*l*)

| Run | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Feed | Reactor | Feed | Reactor | Feed | Reactor | Feed | Reactor |
| PH | 4,13 | 4,84 | 4,06 | 4,91 | 4,14 | 5,05 | 4,16 | 5,00 |
| Alk $H_2CO_3$* | $5,57x10^{-8}$ | $2,79x10^{-7}$ | $4,98x10^{-8}$ | $3,48x10^{-7}$ | $5,98x10^{-8}$ | $4,72x10^{-7}$ | $6,23x10^{-8}$ | $4,20x10^{-7}$ |
| Alk $H_2PO_4^{2-}$ | $-6,58x10^{-6}$ | $2,72x10^{-7}$ | $-6,80x10^{-6}$ | $1,11x10^{-6}$ | $-7,86x10^{-6}$ | $8,63x10^{-7}$ | $-6,01x10^{-6}$ | $1,23x10^{-6}$ |
| Alk $NH_3$ | -0,007496 | -0,000143 | -0,006925 | -0,000214 | -0,010566 | -0,000571 | -0,009138 | -0,000500 |
| Alk Hac | 0,009168 | ? | 0,008422 | ? | 0,012329 | ? | 0,011381 | ? |
| Alk $H_2O$ | $-8,45x10^{-5}$ | $-1,61x10^{-5}$ | $-9,92x10^{-5}$ | $-1,38x10^{-5}$ | $-8,37x10^{-5}$ | $-9,99x10^{-6}$ | $-7,95x10^{-5}$ | $-1,12x10^{-5}$ |
| $S_{ALK}$ | 0,001581 | 0,001581 | 0,001391 | 0,001391 | 0,001671 | 0,001671 | 0,002157 | 0,002157 |
| Calculated Alk HAc | 0,001740 | | 0,001617 | | 0,002251 | | 0,002666 | |
| HAc consumed (mg/*l*) | 2428 | | 2562 | | 3203 | | 2798 | |
| Calculated $S_A$ | 182 | | 158 | | 197 | | 242 | |

TABLE 4.6 - Difference in measured and calculated HAc values (mg/*l*)

| Run | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Measured Hac consumed | 2380 | 2250 | 3220 | 2840 |
| Calculated HAc consumed | 2428 | 2562 | 3203 | 2798 |
| Difference | 48 | 312 | -17 | -42 |
| Difference per measured % | 2 | 14 | -0,5 | -1,5 |
| Measured $S_A$* | 230 | 470 | 180 | 200 |
| Calculated $S_A$ | 182 | 158 | 197 | 242 |
| Difference | 48 | 312 | -17 | -42 |

* GC results expressed as HAc.

**Theory application**

The next obvious question is whether the developed theory and equations will hold true for the actual effluent. The subsystem and solution alkalinities with changed reference species and the alkalinity yields, $Y_{ALK}(m)$, were calculated using Eq. 30 and are shown in Tables 4.7 and 4.8 The theoretical alkalinity yields, $Y_{ALK}(t)$, were calculated with Eq. 19 and are shown in Table 4.9.

The COD of the biomass was calculated to determine $f_s$, with COD/TSS = 1,20 gCOD/gTSS, determined by Kühn (1989) for *Geotrichum spp*. The COD differences were calculated by the difference between the measured COD in the feed and the calculated effluent COD, using the measured effluent SCFA and the average ratio of the feed COD : SCFA (1,385).

The alkalinity yields indicate relative small differences, but all the theoretical alkalinity yield values are bigger than the measured values, with margins bigger than that for the laboratory test work. This indicates a possible deviation from the calculation method applied in calculating the theoretical alkalinity yield.

TABLE 4.7 - Subsystem and solution alkalinities for alkalinity yield determination

| Run | 1 | | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|---|---|
| Parameter | Feed | Reactor | Feed | Reactor | Feed | Reactor | Feed | Reactor |
| Alk $CO_3^{2-}$ | $-1{,}52 \times 10^{-5}$ | $-1{,}54 \times 10^{-5}$ | $-1{,}65 \times 10^{-5}$ | $-1{,}68 \times 10^{-5}$ | $-1{,}60 \times 10^{-5}$ | $-1{,}65 \times 10^{-5}$ | $-1{,}60 \times 10^{-5}$ | $-1{,}64 \times 10^{-5}$ |
| Alk $H_3PO_4$ | 0,000768 | $6{,}48 \times 10^{-5}$ | 0,000671 | 0,000195 | 0,000993 | $9{,}77 \times 10^{-5}$ | 0,000801 | 0,000163 |
| Alk $NH_4^+$ | $1{,}05 \times 10^{-7}$ | $1{,}00 \times 10^{-8}$ | $6{,}72 \times 10^{-8}$ | $1{,}45 \times 10^{-8}$ | $1{,}34 \times 10^{-7}$ | $5{,}71 \times 10^{-8}$ | $1{,}21 \times 10^{-7}$ | $4{,}45 \times 10^{-8}$ |
| Alk $Ac^-$ | -0,034294 | -0,001636 | -0,036873 | -0,003015 | -0,044289 | -0,000938 | -0,039243 | -0,001128 |
| Alk $H_2O$ | $-8{,}45 \times 10^{-5}$ | $-1{,}61 \times 10^{-5}$ | $-9{,}92 \times 10^{-5}$ | $-1{,}38 \times 10^{-5}$ | $-8{,}37 \times 10^{-5}$ | $-9{,}99 \times 10^{-6}$ | $-7{,}95 \times 10^{-5}$ | $-1{,}12 \times 10^{-5}$ |
| $S_{ALK}$ | -0,033626 | -0,001603 | -0,036317 | -0,002851 | -0,043396 | -0,000867 | -0,038537 | -0,000993 |
| $S_{ALK}-S_{ALK0}$ | 0,032023 | | 0,033466 | | 0,042529 | | 0,037544 | |

TABLE 4.8 - Measured alkalinity yield, $Y_{ALK}$(m)

| Run | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| X (gSS/*l*) | 3,55 | 4,27 | 4,62 | 4,63 |
| $Y_{obs}$ (gSS/gVFA)* | 0,367 | 0,337 | 0,361 | 0,344 |
| $i_s$ | 1/60 | 1/60 | 1/60 | 1/60 |
| SRT | 11,3 | 17,9 | 12,3 | 15,3 |
| HRT | 2,7 | 3,2 | 3,0 | 3,1 |
| $Y_{ALK}$ (m) | 0,83 | 0,89 | 0,82 | 0,83 |

* Biomass in waste stream and in effluent taken into account.

TABLE 4.9 - Theoretical alkalinity yield, $Y_{ALK}$(t)

| Run | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Effluent COD (g/*l*) | 0,31 | 0,65 | 0,25 | 0,28 |
| $f_s$ | 0,35 | 0,28 | 0,34 | 0,31 |
| $Y_{ALK}$ (t) | 0,86 | 0,89 | 0,86 | 0,88 |
| Difference % * | +3,6 | +0,4 | +5,7 | +5,9 |

* Difference between $Y_{ALK}$ (m) (Table 4.8) and $Y_{ALK}$ (t) per $Y_{ALK}$ (m).

**The theoretical alkalinity yield**

The theoretical alkalinity yield is calculated using half reactions that depends on the electron donor, acceptor and cell synthesis reactions. Referring to Table 4.1 with the typical substrate composition, it is given that the electron donor species are mainly acetic-, propionic- and butyric acids, with approximate percentage contributions of 70%, 20% and 10%, respectively. Considering the half reactions and the theoretical alkalinity yield for each, a composite equation similar to Eq. 19 can be

derived. The electron acceptor and cell synthesis reactions stayed the same as in Chapter II. The electron donor half reactions are:

$$\text{acetic acid, 70\%} : {}^{1}/_{8}\ CH_3COO^- + {}^{3}/_{8}\ H_2O = {}^{1}/_{8}\ CO_2 + {}^{1}/_{8}\ HCO_3^- + H^+ + e^-$$

$$\text{propionic acid, 20\%} : {}^{1}/_{14}\ CH_3CH_2COO^- + {}^{5}/_{14}\ H_2O = {}^{1}/_{7}\ CO_2 + {}^{1}/_{14}\ HCO_3^- + H^+ + e^-$$

$$\text{butyric acid, 10\%} : {}^{1}/_{20}\ CH_3CH_2CH_2COO^- + {}^{7}/_{20}\ H_2O = {}^{3}/_{20}\ CO_2 + {}^{1}/_{20}\ HCO_3^- + H^+ + e^-$$

and an equivalent equation to Eq. 19 is:

$$Y_{ALK} = 1 - f_s\ ({}^{8}/_{20} * 0{,}7 + {}^{14}/_{20} * 0{,}2 + {}^{20}/_{20} * 0{,}1)$$

$$= 1 - f_s\ {}^{10{,}4}/_{20}$$

Calculating the theoretical alkalinity yields using this equation give respective values of 0,82; 0,86; 0,82 and 0,84 for Runs 1, 2, 3 and 4, with differences of -1,3; -3,3; +0,7 and +1,4 percent, compared to the measured values. These values indicate a decrease in the differences and result in differences similar to the laboratory results. It shows that the theoretical alkalinity yield needs to be calculated independently from the equilibrium calculations, taking the actual substrate composition into account although HAc is used to represent the SCFAs in the equilibrium calculations. It also indicates that the simplification to represent a mixture of SCFAs by HAc is successful in applying the substitution throughout the calculation process of equilibrium chemistry and modelling. In this application the HAc was the main acid present, however.

**Process adaptation**

In Section 1.3[1] above a hypothesis on the control methodology was given, stating that the biomass concentration will increase to the point where oxygen will be limiting. The biomass concentration should thus increase with an increase in the oxygen supply. This can be noticed in Table 4.10, indicating an increase in biomass concentration from Runs 1 to 2 and 2 to 3 with an increase in the air supply. The biomass concentration should also increase with an increase in the SRT (Grady *et al.* 1999) but stayed the same for Runs 3 and 4. This is unexpected.

To understand the results it is necessary to consider the oxygen supply, uptake and transfer rates, as the process was operated on oxygen limitation. A COD balance was completed to calculate the

[1] *Vide* p85

oxygen uptake (RO), and the oxygen supply was calculated from the air supply rate. The results are shown in Table 4.10.

TABLE 4.10 - Oxygen supply and uptake rates and transfer efficiencies

| Run | V/V* | SRT (h) | X (g/*l*) | COD removal kg/m$^3$h** | RO kg/h | Oxygen supply kg/h | Oxygen transfer % | $r_{so}$ kg/m$^3$h |
|---|---|---|---|---|---|---|---|---|
| 1 | 0,64 | 11,3 | 3,55 | 1,03 | 3,53 | 63,0 | 5,6 | 0,64 |
| 2 | 0,85 | 17,9 | 4,27 | 1,00 | 3,90 | 83,6 | 4,7 | 0,71 |
| 3 | 1,35 | 12,3 | 4,62 | 1,27 | 4,09 | 124,2 | 3,3 | 0,80 |
| 4 | 1,30 | 15,3 | 4,63 | 1,11 | 3,91 | 124,5 | 3,1 | 0,73 |

* V/V: Volume air per volume reactor per minute.

** COD removal per reactor volume per hour.

Considering Runs 3 and 4, an increase in both the biomass concentration and the RO would be expected for a normal bioreactor operation due to the increase in the SRT. With oxygen limitation though, an increase in the biomass concentration will negatively influence oxygen transfer, and with the oxygen supply rate constant, the self-regulating technique acts in a reverse fashion by keeping the biomass concentration approximately constant with the increase in the SRT. This effectively decreases the total active amount of biomass in the process and also the amount of COD removed (Table 4.10). The RO increases with the increase in the SRT, but simultaneously decreases with the associated amount by which the COD removal decreased. The result is a decrease in RO, the oxygen reaction rate, $r_{so}$, and in oxygen transfer. With the effluent COD and SCFA concentrations approximately constant, the pHauxostat decreased the feed concentration from Run 3 to 4, due to the decrease in COD removal rate (Table 4.3).

This demonstrates the complex but natural changes taking place in the pHauxostat. It also indicates the benefit of using the pHauxostat technique that adapts to changes without wastage or effluent quality impairment and can be used to determine optimised oxygen transfer rates and oxygen reaction rates, $r_{so}$.

**Design information**

From the results it can for example be concluded that the increase in air supply from 0,85 to 1,30 $m^3/m^3$.min did not increase $r_{so}$, but only decreased oxygen transfer (comparing Runs 2 and 4 and taking the difference in $\theta_c$ into account). The $r_{so}$ did however increase with the increase in air supply from 0,64 to 0,85 $m^3/m^3$.min. The maximum value for $r_{so}$ will approximately be 0,80 $kg/m^3h$ for a SRT of ca. 12 hours and is already reached at an air supply of 0,85 $m^3/m^3$.min with an oxygen transfer of probably a little bit higher than 4,7%, say ca. 5%. From these results a feasibility study can be done and a full scale plant designed.

The important demonstration is however the confirmation of the expected control methodology.

## 1.7 Conclusions

The results from the demonstration plant demonstrated that the proposed calculation method and theory is applicable and can successfully be applied for a pHauxostat with natural industrial effluent as substrate. It was shown that the pHauxostat with a biomass separator can successfully be operated and may be classified as a Category B pHauxostat and will normally operate on oxygen limitation. The conclusions are summarised below :

- The equilibrium chemistry and the use of subsystem and solution alkalinities in analysing the pHauxostat can successfully be applied with natural effluents as substrate.

- The simplification in the calculations by considering only acetic acid to represent a mixture of SCFA's gave satisfactory results.

- A pHauxostat with biomass separator can be described by the proposed theory and the developed equations represented by Eq. 30:

$$i_s X_{COD} Y_{ALK} (\tau/\theta_c) / Y_{obs} = S_{ALK} - S_{ALK0}$$

- The demonstration plant demonstrated that the pHauxostat control technique results in an easy, stable and reliable operation.

# 2. DEMONSTRATION

## 2.1 Introduction

It is convenient to demonstrate correlations between different parameters with plots. Plots are constructed in this section to visualise and understand the pHauxostat process and its reaction to changes in these parameters. The plots are constructed by using the equations from the previous sections with the assumption of growth kinetics as given in Table 4.11 below:

TABLE 4.11 - Assumed growth kinetics and values for demonstration purposes

| Parameter | Value |
|---|---|
| Y | 0,59* |
| $Y_{TALK}$ | 0,77** |
| $\mu_m$ ($h^{-1}$) | 0,4 |
| $K_s$ (mg COD/*l*) | 50 |
| b ($h^{-1}$) | 0,01 |
| $i_s$ acetic acid (mol/COD) | $^1/_{64}$ |
| V (*l*) | 50 |

* For acetic acid as electron donor from McCarty (1975)

** Calculated with Eq. 21

Heterotrophic growth is assumed with acetic acid as substrate. The Y is calculated by the method proposed by McCarty (1975) with acetic acid the electron donor and oxygen the electron acceptor. The calculated value (0,59) is used in Eq. 21[1] to calculate $Y_{TALK}$ with x = 1 and y = $-^8/_{20}$ . Cell synthesis is assumed with ammonia as nitrogen source (Chapter II[2]). $Y_{TALK}$ was calculated as 0,77.

[1] *Vide* Chapter II p40
[2] *Vide* Chapter II p37

## 2.2 General plots

**Figure 4.3**

The basic principles of growth in a CSTR are also true for the pHauxostat reactor. The Monod equation, Eq. 3[1], and the corresponding HRT (Eq. 6[2]) are plotted in Fig. 4.3. The HRT decreases as the growth rate and the GLN concentration increases.

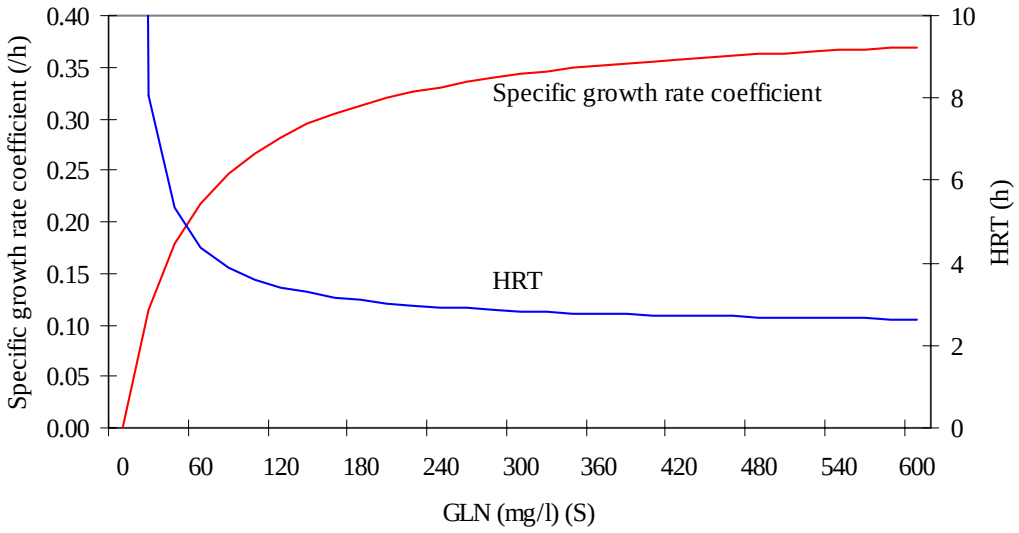


FIG. 4.3 - Monod and HRT

**Figure 4.4**

The residual substrate concentration (normally the GLN concentration) (Eq. 5[2]), the growth rate (Eq. 6[2]) and the biomass concentration (Eq. 4[2]) are plotted against the HRT in Fig. 4.4. The effect of increasing the substrate concentration in the feed can be seen in the increase in the biomass concentration. The residual substrate concentration stays the same for the same HRT because the growth rate does not change and therefore also not the GLN concentration. The decrease in X with longer HRT's is due to a decrease in $Y_{obs}$, as shown in Fig. 4.5.

[1] *Vide* Chapter I p3
[2] *Vide* Chapter I p5

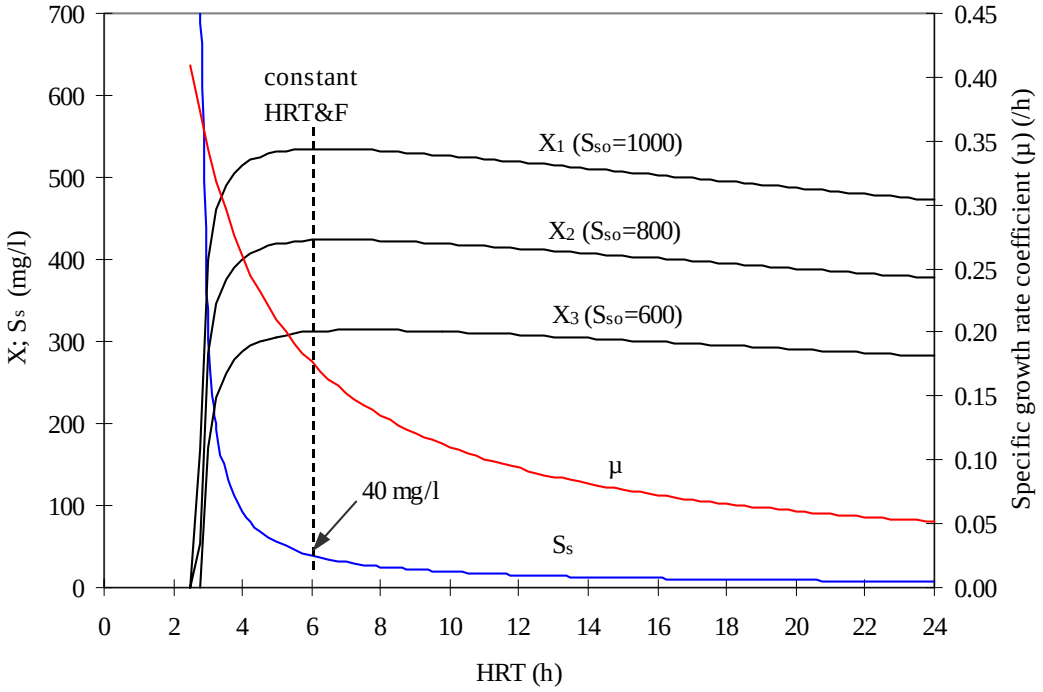


FIG. 4.4 - The change in X with change in $S_{so}$

## 2.3 pHauxostat plots

**Figure 4.5**

In Fig. 4.5 the change in $Y_{obs}$ (Eq. 7[1]) and $Y_{ALK}$ (Eq. 20[2]) as well as the change in its ratio ($i_s Y_{ALK}/Y_{obs}$) are plotted against the HRT. The ratio represents a main portion of the left side of Eqs. 16 and 17[3]. The plots indicate that $Y_{ALK}$ increases as $Y_{obs}$ decreases, and its ratio increase with increase in HRT. $Y_{ALK}$ increases with decrease in $Y_{obs}$ because as less cell material is synthesised, less alkalinity is consumed (refer half reactions). The change in the yield ratio will be different for different substrates and will depend on the value of x, refer Eq. 22[4].

[1] *Vide* Chapter I p5
[2] *Vide* Chapter II p39
[3] *Vide* Chapter II p36
[4] *Vide* Chapter II p40

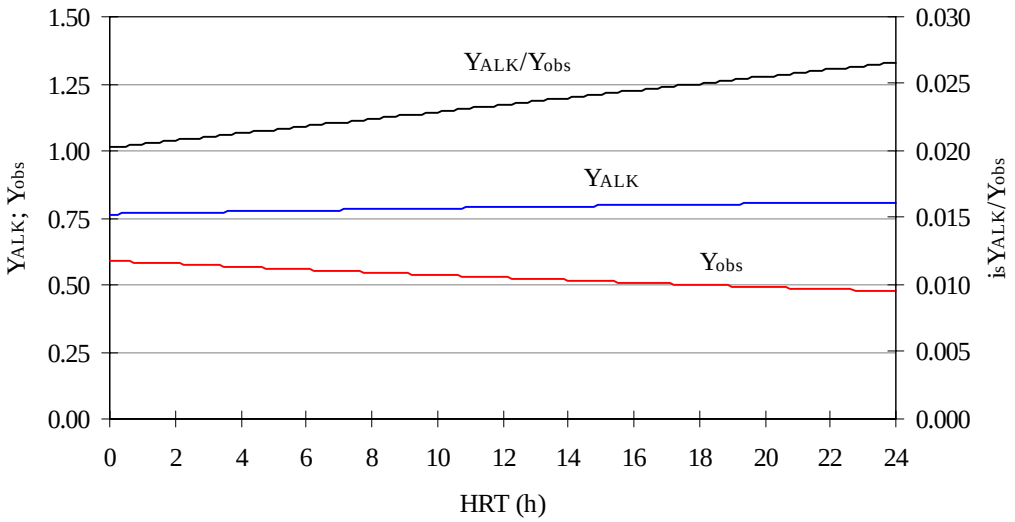


FIG. 4.5 - The change in yields with change in HRT

**Figure 4.6**

The influence of the difference in solution alkalinities between the substrate and the reactor solutions is shown in Fig. 4.6. The HRT decreases with decrease in alkalinity difference with a corresponding increase in residual substrate concentration. For a constant pH set point, the alkalinity difference may be decreased by addition of base or increased by addition of acid to the substrate, as indicated in the plot. The effect of an increased substrate concentration is also shown, indicating that the pHauxostat can only operate within a certain range of alkalinity differences. It is also possible to operate the pHauxostat at a constant feed rate when changing the substrate concentration by manipulating the alkalinity difference, moving on the shown stippled line at constant HRT. The GLN concentration is thereby kept constant. The plot was constructed by using Eq. 16[1] (or 17). It also indicates that the pHauxostat is least sensitive to alkalinity changes at short HRT's and increase in sensitivity as the HRT increases. This implies that in this case the control of the process should be better in the faster growth rate range, than in the slower growth rate range. Fluctuations in the substrate alkalinity, especially in the slow growth rate range, may clearly have a major impact on process control, which will be true for pHauxostats in general.

[1] *Vide* Chapter II p36

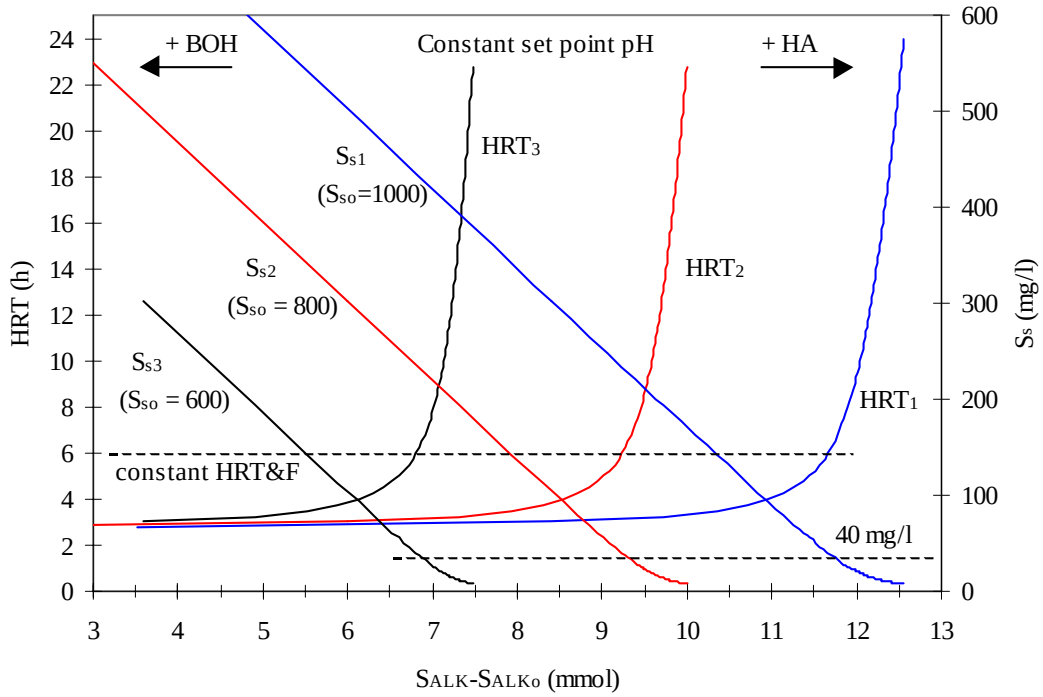


FIG. 4.6 - The change in HRT and $S_s$ with change in alkalinity differences

**Figure 4.7**

Fig. 4.7 is a plot similar to Fig. 4.4 with inclusion of the alkalinity difference (Eq. 16[1] or 17) and the alkalinity production rate, $r_{ALK}$, (Eq. 15[1]), but for a single substrate concentration. The alkalinity production rate is a maximum near washout and decreases with an increase in the HRT. The feed rate is plotted for a reactor volume of 50 *l*, which was arbitrary chosen to fit into the plot area. It indicates that the flow rate will increase with addition of base to the substrate, with an obvious decrease in the HRT.

**Figure 4.8**

The same parameters as in Fig. 4.7 are plotted in Fig. 4.8 but against the alkalinity difference, giving the same answer but from a different angle. Once the substrate, the growth kinetics and the alkalinity yield for a specific process is known, then similar plots as in Figs. 4.7 and 4.8 may be done to quantify the process and help in decision making.

[1] *Vide* Chapter II p36

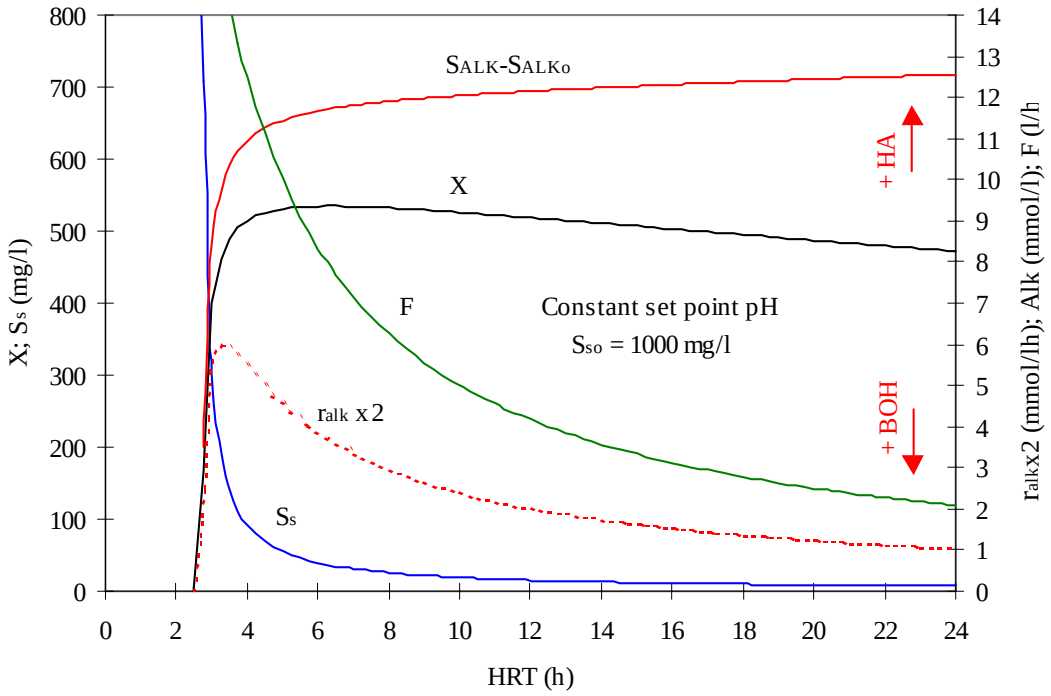


FIG. 4.7 - The pHauxostat parameters plotted against HRT

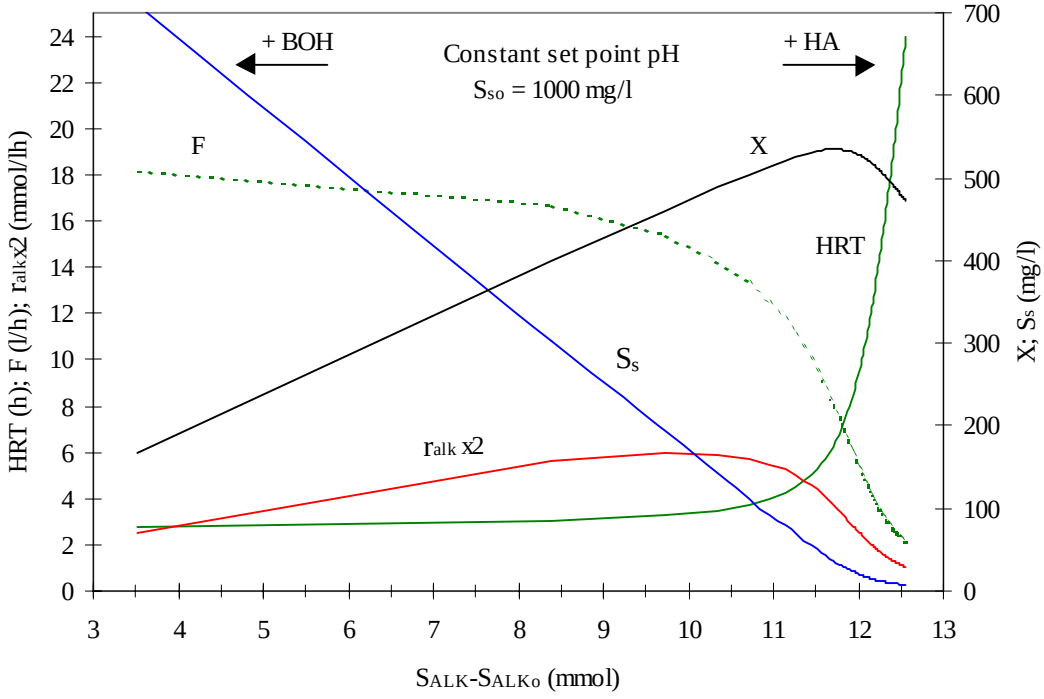


FIG. 4.8 - The pHauxostat parameters plotted against alkalinity difference

## 2.4 Explanation

### Category A pHauxostats

Applying the control technique for Category A pHauxostats results in a GLN concentration and alkalinity difference due to the selection of the pH set point for a given solution. The growth rate and the HRT are thereby determined and given in Fig. 4.4. In Fig. 4.8 the resulting flow rate and biomass concentration for the given solution is shown plus the alkalinity difference and alkalinity production rate. For a given pH set point the process may be manipulated by adding base or acid to the substrate with a corresponding change in the GLN concentration and in the alkalinity difference. The GLN concentration and the change need to be calculated by using the developed equilibrium chemistry with alkalinities. A second manipulation is possible by changing the substrate concentration with the effect given in Figs. 4.4 and 4.6. An equivalent plot to Fig. 4.7 or 4.8 can then be done for the selected substrate concentration.

### Category B pHauxostats

For Category B pHauxostats the plots are the same but the GLN used in the graphs will probably not be the residual carbon substrate. Plots similar to Figs. 4.7 and 4.8 can be done for a specific nutrient feed rate and will differ for different feed rates as in the previous case for different substrate concentrations. As described in the control methodology for Category B pHauxostats, the alkalinity production rate is controlled by the GLN feed rate, and for a given alkalinity difference it results in a specific feed rate and HRT, which form part of Figs. 4.7 and 4.8.

The plots for the pHauxostat with recycle will also be similar to the above graphs but with the HRT exchanged for the SRT and plots done for a constant HRT. Each HRT will result in a set of plots.

# BIBLIOGRAPHY


Agrawal, P. and Lim, H.C. 1984. Analyses of various control schemes for continuous bioreactors. *Adv. Biochem. Eng. / Biotechnol.,* 30 : 61 – 90.

Aiba, S., Humphrey, A.E. and Millis, N.F. 1965. *Biochemical Engineering.* New York : Academic Press.

Augustyn, M.P. 1995. *An evaluation of process operating parameters for Geotrichum biomass production on $C_2 – C_5$ monocarboxylic acids in a continuous airlift reactor with internal recycle*. MSc dissertation, Univ. of the Free State, Bloemfontein.

Austermann-Haun, U., Seyfried, C.F., Kunst, S., Brockmann, M., Bänziger, W. and Rosenwinkel, K.H. 1994. Anaerobic pretreatment in campaign industries. *Seventh International Symposium on Anaerobic Digestion. Cape Town, Jan. 23-27.*

Bailey, J.E. and Ollis, D.F. 1986. *Biochemical Engineering Fundamentals.* 2nd edition. New York : McGraw-Hill.

Benefield, L.D., Judkins, J.F. and Weand, B.L. 1982. *Process chemistry for water and wastewater treatment.* Englewood Cliffs, New Jersey : Prentice-Hall.

Blanch, H.W. and Clark, D.S. 1997. *Biochemical Engineering.* New York : Marcel Dekker.

Brierly, M.R. and Steel, R. 1959. Agitation in submerged fermentation. Part 2. Effect of solid disperse phase on oxygen adsorption in a fermenter. *Appl. Microbiol.,* 57(7), as quoted by Aiba *et al*., 1965.

Brune, G., Schoberth, S.M. and Sahm, H. 1982. Anaerobic treatment of an industrial wastewater containing acetic acid, furfural and sulphite. *Process Biochem.,* May/June, 35 : 20 – 24.

Bryson, V. and Szibalski, W. 1952. Microbial selection. *Science,* 116 : 45 –51.

Chisholm, S.W., and Stross, R.G. and Nobbs, P.A. 1975. *J. Phycol.,* 11 : 367 – 373.

Choi, J. and Lee, S.Y. 1999. High-level production of poly (3-Hydroxybutyrate-co-3-Hydroxyvalerate) by fed-batch culture of recombinant *Escherichia coli. Appl. Environ. Microbiol.,* 65(10) : 4363 – 4368.

CSIR. 1970a. *Special Report CHEM 153.* Council for Scientific and Industrial Research, Pretoria.

CSIR. 1970b. *Special Report CHEM 214.* Council for Scientific and Industrial Research, Pretoria.

Defrance, M.B., Elmaleh, S., Ghommidh, C. and Navarro, J.M. 1996. *Wat. Res.,* 30(10) : 2526 – 2529.

Demirer, G.N. and Speece, R.E. 1999. Inhibitory effects and biotransformation of acrylic acid in computer-controlled pH-stat CSTRs. *Biotechnol. Bioeng.,* 62(2) : 200 – 207.

Demirer, G.N. and Speece, R.E. 2000. Comparison of anaerobic acrylic acid biotransformation in single- and two-stage pH-stat completely stirred tank reactor systems. *Wat. Environ. Res.,* 72(1) : 84 – 89.

Driessen, F.M., Ubbels, J. and Stadhouders, J. 1977. Continuous manufacture of yogurt. I. Optimal conditions and kinetics of the prefermentation process. *Biotechnol. Bioeng.,* 14 : 821–839.

Duetz, W.A., Winson, M.K., van Andel, J.G. and Williams, P.A. 1991. Mathematical analysis of catabolic function loss in a population of *Pseudomonas putida* mt – 2 during non – limited growth on benzoate. *J. Gen. Microbiol.,* 137(6) : 1363 – 1368.

Du Preez, J.C. and Van der Walt, J.P. 1983. Fermentation of D – xylose to ethanol by a strain of *Candida shehatae. Biotechnol. Lett.,* 5(5) : 357 – 362.

Edwards, V.H., Ko, R.C. and Balogh, S.A.1972. Dynamics and control of continuous propagators to subject substrate inhibition. *Biotechnol, Bioeng.,* 14 : 939 – 974.

Fiebig, R. and Dellweg, H. 1985. Comparison between the process performance of an UASB – reactor and an UASB-fixed film-combination with an acetic enrichment culture. *Biotechnol. Lett.,* 7(7) : 487 – 492.

Fraleigh, S.P., Bungay, H.R. and Clesceri, L.S. 1989. Continuous culture, feedback control and auxostats. *Trends Biotechnol.,* 7 : 159 – 164.

Fuld, G.J. and Dunn, C.G. 1957. New process control applications in fermentation. *Ind. Eng. Chem.,* 49 (8) : 1215 – 1220.

Gaudy, A.F. and Gaudy, E.T. 1980. *Microbiology for Environmental Scientists and Engineers.* New York : McGraw-Hill.

Gerhardt, P., Murray, R.G.E., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R. and Phillips, G.B. 1981. *Manual of methods for general bacteriology.* Washington : American Society for Microbiology.

Girginov, T. 1965. *Lebensm. Ind.,* 12 : 263, cited by Driessen *et al.,* 1977.

Gottschal, J.C. 1990. Different types of continuous culture in ecological studies. *Methods in Microbiol.,* 22 : 87 – 124.

Grady, C.P.L. and Lim, H.C. 1980. *Biological Wastewater treatment, theory and application.* New York : Marcel Dekker.

Grady, C.P.L., Daigger, G.T. and Lim, H.C. 1999. *Biological Wastewater Treatment.* New York : Marcel Dekker.

Herbert, D., Elsworth, R. and Telling, R.C. 1956. The continuous culture of bacteria; a theoretical and experimental study. *J. Gen. Microbiol.,* 14 : 601 – 622.

Herbert, D. 1959. Some principles of continuous culture. *Recent progress in microbiology : VI International Congress for Microbiology. Stockholm, 1959.*

Hospodka, J. 1966a. Oxygen-absorption rate-controlled feeding of substrate into aerobic microbial cultures. *Biotechnol. Bioeng.,* 8 : 117 –134.

Hospodka, J., Málek, I. (ed.) and Fencl, Z. (ed.). 1966b. Theoretical and methodological basis of continuous culture of microorganisms. New York : Academic Press.

IAWPRC. 1986. *Activated Sludge Model No.1.* International Association on Water Pollution Research and Control, London.

IWA. 2000. *Activated Sludge Models ASM 1, ASM 2, ASM 2D and ASM 3.* Londen : IWA Publishing.

Kistner, A., Kornelius, J.H. and Miller, G.S. 1983. Kinetic measurements on bacterial cultures growing on fibres. *S.Afr. J. Anim. Sci.*,13(3) : 217 – 220.

Kobayashi, G., Tanaka, K., Itoh, H., Tsuge, T., Sonomoto, K. and Ishizaki, A. 2000. Fermentative production of P(3HB-co-3HV) from propionic acid by *Alcaligenes eutrophus* in fed – batch culture with pH-stat continuous substrate feeding method. *Biotechnol. Lett.*, 22(13) : 1067 – 1069.

Kühn, A.L. and Pretorius W.A. 1988. Use of the crossflow-microscreen technique for SCP production form dilute substrates. *Appl. Microbiol. Biotechnol.*, 27 : 593 – 600.

Kühn, A.L. and Pretorius, W.A. 1989. Biologiese suiwering van nywerheidsuitvloeisel met gelyktydige produksie van enkelselproteïen. *SA Tyd. Chem. Ing.*, 1(1) : 14 – 22.

Kühn, A.L. 1989. *Enkelselproteïenproduksie uit monokarboksielsuur afvalwater*. PhD thesis, Univ. of Pretoria, Pretoria.

Levenspiel, O. 1999. *Chemical Reaction Engineering*. 3rd edition. New York : John Wiley.

Lim, D. 1998. *Microbiology.* New York : McGraw-Hill.

Linder, P.W., Torrington, R.G. and Williams, D.R. 1984. *Analysis Using Glass Electrodes.* Belfast : Open University Press.

Loewenthal, R.E. and Marais, G.v.R. 1976. *Carbonate Chemistry of Aquatic Systems : Theory & Application.* Michigan : Ann Arbor Science.

Loewenthal, R.E., Ekama, G.A. and Marais, G.v.R. 1989. Mixed weak acid/base systems, Part I – Mixture characterisation. *Water SA,* 15(1) : 3 – 23.

Loewenthal, R.E., Wentzel, M.C., Ekama, G.A.and Marais, G.v.R. 1991. Mixed weak acid/base systems, Part II : Dosing estimation, aqueous phase. *Water SA,* 17(2) : 107 – 122.

MacBean, R.D., Hall, R.J. and Linklater, P.M. 1979. Analysis of pH-stat continuous cultivation and the stability of the mixed fermentation in continuous yogurt production. *Biotechnol. Bioeng.*, 21: 1517 – 1541.

Martin, A.M., Goddard, S. and Bemister, P. 1993. Production of *Candida utilis b*iomass as aqua – culture feed. *J. Sci. Food Agric.*, 61 : 363 – 370.

Martin, G.A. and Hempfling, W.P. 1976. A method for the regulation of microbiol population density during continuous culture at high growth rates. *Arch. Microbiol.*, 107 : 41 – 47.

McCarty, P.L. 1975. Stoichiometry of Biological reaction. *Prog. Wat. Technol.*, 7(1) : 157 – 172.

McCarty, P.L. and Mosey, F.E. 1991. Modelling of anaerobic digestion processes. *Wat. Sci. Technol.*, 24(8) : 17 – 33.

Menawat, A.S. and Balachander, J. 1991. Alternate control structures for chemostat. *AIChE J.*, 37(2) : 302 – 306.

Monod, J. 1942. *Recherces sur la croissance des cultures bacteriennes* Paris : Hermann and Cie, cited by Monod, 1949.

Monod, J. 1949. The growth of bacterial cultures. *Ann. Rev. of Microbiol.*, 3 : 371 – 394.

Monod, J. 1950. La technique de culture continue; théorie et applications. *Ann. Inst. Pasteur, Paris.* 79 : 390, cited by Herbert *et al.*, 1956.

Moosbrugger, R.E., Wentzel, M.C., Ekama, G.A. and Marais, G,v.R. 1993a. Alkalinity measure – ment : Part 1- A 4 pH point titration method to determine the carbonate weak acid/base in an aqueous carbonate solution. *Water SA*, 19(1) : 11 – 22.

Moosbrugger, R.E., Wentzel, M.C., Ekama, G.A. and Marais, G.v.R. 1993b. Alkalinity measure – ment : Part 2- A 4 pH point titration method to determine the carbonate weak acid/base in aqueous solutions containing other weak acid/bases of known concentrations. *Water SA*, 19(1) : 23 – 28.

Moosbrugger, R.E., Wentzel, M.C., Ekama, G.A. and Marais, G.v.R. 1993c.Lauter tun (brewery) waste in UASB systems – Feasibility, alkalinity requirements and pH control. *Water SA*, 19(1) : 41 – 52.

Moosbrugger, R.E., Wentzel, M.C., Ekama, G.A. and Marais, G.v.R. 1993d. Weak acid/bases and pH control in anaerobic systems – A review. *Water SA,* **19**(1) : 1 –10.

Moosbrugger, R.E., Wentzel, M.C., Loewenthal, R.E., Ekama, G.A. and Marais, G.v.R. 1993. Alkalinity measurement : Part 3- A 5 pH point titration method to determine the carbonate and SCFA weak acid/bases in aqueous solution containing also known concentrations of other weak acid/base. *Water SA*, 19(1) : 29 – 40.

Müller, R.H., Simon, D., Große, H.J. and Babel, W. 1997. Substrate inhibition under stationary growth conditions – nutristat experiments with *Ralstonia eutropho* JMP 134 during growth on phenol and 2,4 – dichlorophenoxyacetate. *Appl. Microbiol. Biotechnol.,* 48(5) : 648 – 655.

Musvoto, E.V., Wentzel, M.C., Loewenthal, R.E. and Ekama, G.A. 1997. Kinetic – based model for mixed weak acid/base systems. *Water SA,* 23(4) : 311 – 322.

Nel, L.H., Britz, T.J. and Lategan, P.M. 1985. The effect of trace elements on the performance efficiency of an anaerobic fixed film reactor treating a petrochemical effluent. *Water SA,* 11(3) : 107 – 110.

Nguyen, A – L., Duff, S.J.B. and Sheppard, J.D. 2000. Application of feedback control based on dissolved oxygen to a fixed – film sequencing batch reactor for treatment of brewery wastewater. *Wat. Environ. Res.,* 72(1) : 75 –83.

Novick, A. and Szilard, L. 1950. Experiments with the chemostat on spontaneous mutations of bacteria. *Proc. Nat. Acad. Sci*., 36 : 708.

Olsson, G. and Newell, B. 1999. *Wastewater Treatment Systems. Modelling, Diagnosis and Control.* London : IWA Publishing.

Oltmann, L.F., Schoenmaker G.S., Reijnders, W.N.M. and Stouthamer, A.H.1978. Modification of the pH-auxostat culture method for the mass cultivation of bacteria. *Biotechnol. Bioeng.,* 20 : 921 – 925.

Pretorius, W.A. 1987. A conceptual basis for microbiol selection in biological wastewater treatment. *Wat. Res.,* 21(8) : 891 – 894.

Pretorius, W.A. 1995. pH-Controlled feed-on-demand for high-rate anaerobic systems. *Wat. Sci. Tech.,* 30(8) : 1 – 8.

Ratledge, C. and Kristiansen, B.(eds). 2001. *Basic Biotechnology.* 2nd edition, Cambridge : Cambridge University Press.

Rice, C.W. and Hempfling, W.P. 1978. Oxygen – limited continuous culture and respiratory energy conservation in *Escherichia coli. J. Bacteriol.,* 134(1) : 115 – 124.

Rice, C.W. and Hempfling, W.P. 1985. Nutrient – limited continuous culture in the phauxostat. *Biotechnol. Bioeng.,* 27 : 187 – 191.

Ross, W.R. and Louw, J.M. 1987. Monitoring and control of anaerobic digestion. *Water SA,* 13(4) : 193 – 196.

Rutgers, M., Bogte, J.J., Breure, A.M. and Van Andel, J.G. 1993. Growth and enrichment of pentachlorophenol – degrading microorganisms in the nutristat, a substrate concen – tration – controlled continuous culture. *Appl. Environ. Microbiol.,* 59(10) : 3373 –3377.

Rutgers, M., Gooch, D.D., Breure, A.M. and Van Andel, J.G. 1996. Assessment of inhibition kinetics of the growth of strain P5 on pentachlorophenol under steady – state conditions in a nutristat. *Arch. Microbiol.*, 165(3) : 194 – 200.

Rutgers, M., Van Bommel, S., Breure, A.M., Van Andel, J.G. and Dentz, W.A. 1998. Effect of pH on the toxicity and biodegradation of pentachlorophenol by *Sphingomonas* sp. strain P5 in nutristat culture. *Environ. Toxicol. Chem.*, 17(5) : 792 – 797.

Sam-Soon, P.A.L.N.S., Loewenthal, R.E., Wentzel, M.C., Moosbrugger, R.E.and Marais, G.v.R. 1991. Effects of a recycle in upflow anaerobic sludge bed (UASB) systems. *Water SA*, 17(1) : 37 – 46.

Sawyer, C.N., McCarty, P.L. and Parkin, G.F. 1994. *Chemistry for Environmental Engineering.* 4th edition. New York : McGraw-Hill.

Schulze, D., Fiebig, R. and Dellweg, H. 1988. Development of granular sludge in the UASB-treatment of model waste waters containing gelatine. *Biotechnol. Lett.*, 10(5) : 319 – 324.

Šestáková, M. 1979. Growth of *Candida utilis* on a Mixture of Monosaccharides, Acetic Acid and Ethanol as a Model of Waste Sulphite liquor. *Folia Microbiol.*, 24 : 318 – 327.

Snoeyink, V.L. and Jenkins, D. 1980. *Water Chemistry.* New York : John Wiley.

Sowers, K.R., Nelson, M.J. and Ferry, J.G. 1984. Growth of Acetotrophic, Methane – producing Bacteria in a pH Auxostat, *Cur. Microbiol.*, 11 : 227 – 230.

Speece, R.E. 1996. *Anaerobic Biotechnology : For Industrial Wastewaters.* Nashville, Tennessee : Archae Press.

*Standard Methods for the Examination of Water and Wastewater.* 1980. 15th edition. New York : APHA.

*Standard Methods for the Examination of Water and Wastewater.* 1995. 19th edition. Washington : APHA.

Stouthamer, A.H. and Bettenhaussen, C.W. 1976. Energetic Aspects of Anaerobic Growth of *Aerobacter aerogenes* in Complex Medium. *Arch. Miciobiol.*, 111 : 21 –23.

Stumm, W. and Morgan, J. 1970. *Aquatic Chemistry.* New York : John Wiley.

Stumm, W. and Morgan, J. 1981. *Aquatic Chemistry. An Introduction Emphasizing Chemical Equilibria in Natural Waters.* 2nd edition. New York : John Wiley.

Sugimoto, T., Tsuge, T., Tanaka, K. and Ishizaki, A. 1999. Control of Acetic Acid Concentration by pH-Stat Continuous Substrate Feeding in Heterotrophic Culture Phase of Two-Stage Cultivation of *Alcaligenes eutrophus* for Production of P(3HB) from $CO_2$, $H_2$ and $O_2$ under non-Explosive Conditions. *Biotechnol. Bioeng.*, 62(6) : 625 – 631.

Suzuki, T., Yasuda, T., Yamané, T. and Shimizu, S. 1986. Detection and automatic control of ammonium ion concentration in microbial culture with an ammonium ion selective electrode. *J. Ferment. Technol.,* 64(1) : 63 – 70.

Tsuge, T., Tanaka, K., Shimoda, M. and Ishizaki, A. 1999. Optimization of L – lactic acid feeding for the production of poly – D – 3 – hydroxybutyric Acid by *Alcaligenes eutrophus* in fed – batch culture. *J. Biosci. Bioeng.,* 88(4) : 404 – 409.

Van der Westhuizen, H. 1993. *Seleksie van spesifieke organismes met 'n mikrosif-gebaseerde mikrobiese seleksieproses*. MSc dissertation, Univ. of Pretoria, Pretoria.

Watson, T.G. 1969. Steady state operation of a continuous culture at maximum growth rate by control of carbon dioxide production. *J. Gen. Microbiol.,* 59 : 83 – 89.

Watson, T.G. 1972. The present status and future prospects of the turbidostat. *J. Appl. Chem. Biotechnol.,* 22 : 229 – 243.

Weast, R.C. (ed). 1974. *Handbook of Chemistry and Physics.* 55$^{th}$ edition. Cleveland, Ohio : CRC Press.

Wilkowske, H.H. and Fouts, E.L. 1958. Continuous and automatic propagation of dairy cultures. *J. Diary Sci.* 41 : 49 – 56.

WRC. 1986. *Softening and Stabilization of Municipal Waters.* Pretoria : Water Research Commission.

WRC. 1992. *Simple titration procedures to determine $H_2CO_3^*$ alkalinity and short – chain fatty acids in aqueous solutions containing known concentrations of ammonium, phosphate and sulphide weak acid/bases.* Pretoria : Water Research Commission.

Zhao, Y. and Skogestad, S. 1997. Comparison of various control configurations for continuous bioreactors. *Ind. Eng. Chem. Res.,* 36 : 697 – 705.

# APPENDIX A : GROWTH KINETICS AND BIOREACTOR MODELLING

## 1. Growth kinetics

Growth may be described through catabolic and anabolic pathways by which cell material is synthesised with an associated electron exchange (Lim 1998). In short, substrate is utilised to derive energy, building blocks (nutrients) and reducing power (for electron exchange) from it, with an ultimate transfer of electrons to a terminal electron acceptor. Biomass is produced from these products. Combined, substrate is utilised or consumed and biomass is produced, with a proportionally factor, **the true growth yield (Y)**, coupling the two overall biochemical reactions. The **observed growth yield ($Y_{obs}$)** is less than the true growth yield, as Y is defined as yield without any maintenance energy taken into account. $Y_{obs}$ decreases as the maintenance energy gets proportionally bigger (Grady *et al.* 1999). Growth may be expressed as :

$$r_{XB} = -Yr_S \qquad \text{(A1)}$$

with **$r_{XB}$ the rate of biomass production** and **$r_s$ the rate of substrate consumption** with Y the true growth yield, all expressed in units of chemical oxygen demand (COD). The rate of biomass production or the growth rate can be expressed as a first-order equation:

$$r_{XB} = \mu X_B \qquad \text{(A2)}$$

with **$\mu$ the specific growth rate coefficient** and **$X_B$ the active biomass concentration**. Combining Eqs. A1 and A2 gives:

$$r_s = -\mu X_B / Y \qquad \text{(A3)}$$
$$= -(\mu/Y) X_B$$

$\mu/Y$ may be described as the specific substrate consumption rate.

Monod (1949) proposed an empirical equation describing the inter relationship between growth rate and substrate concentration and can be expressed as:

$$\mu = \mu_m S_s / (K_s + S_s) \qquad \text{(A4)}$$

where $\mu_m$ **is the maximum specific growth rate**, $S_s$ **the substrate concentration** and $K_s$ **the half-saturation coefficient for substrate**, which is the substrate concentration at half maximum specific growth rate. The equation is demonstrated in Chapter I, Fig. 1.1.

The substrate concentration represents the growth limiting nutrient concentration which can be the carbon source, the electron donor, the electron acceptor, or any other factor needed by the organism for growth (Grady *et al*. 1999). The specific growth rate increases as the growth limiting nutrient increases up to the maximum specific growth rate. The equation is generally accepted in literature as a good description of the relationship. The equation is also acceptable for the growth limiting nutrient to be measured in units of COD (Gaudy & Gaudy 1980).

The last biochemical process to describe is decay. Decay is the loss of biomass by predation and lysis for example. It is described by a first order expression similar to growth:

$$r_{XD} = -bX_B \qquad \text{(A5)}$$

with **b the decay coefficient** and $\mathbf{r_{XD}}$ **the reaction rate of biomass decay**.

## 2. Bioreactor modelling : The chemostat

Shown in Fig. A1 is a chemostat or CSTR with an influent and effluent stream and constant volume. Complete mixing is done by mechanical stirrer and/or gas mixing by the gas supplied for aeration.

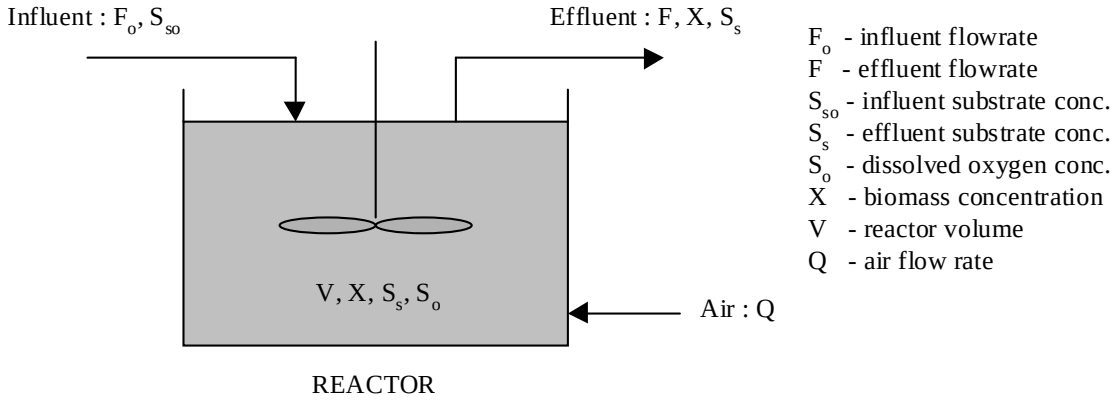


FIG. A1 - The chemostat or CSTR

The CSTR and its modelling is well described by Grady & Lim (1980) and may be explained by completing mass balances over the control volume, taken as the reactor volume (V), on; (i) substrate, (ii) biomass and (iii) COD.

i) On substrate:

$$V.\, dS / dt = F_o.S_{so} - F.S_s + r_s.V \qquad (A6)$$

where $F_o$ and F are the volumetric flow rates for the influent and effluent and $S_{so}$ and $S_s$ the influent and effluent concentrations in COD, respectively. For steady state the equation simplifies to:

$$- r_s = (F/V)\ (S_{so} - S_s) \qquad (A7)$$

The **mean hydraulic residence time** (HRT) with symbol **τ**, is the inverse of the **dilution rate, D**, with:

$$\tau = V/F = 1/D \qquad (A8)$$

Combining Eqs. A3 and A7 and replacing with A8, gives :

$$(F/V)\ (S_{so} - S_s) = \mu X_B/Y$$
$$\therefore \quad X_B = Y(S_{so} - S_s) / \mu\tau \qquad (A9)$$

ii) On biomass: Completing a mass balance on active biomass concentration at steady state and using Eqs. A2, A5 and A8 with no biomass in the influent :

$$0 - FX_B + r_{XB}V + r_{XD}V = 0$$
$$\therefore \quad - X_BV / \tau + \mu X_BV - bX_BV = 0$$
$$\therefore \quad \mu = 1/\tau + b \qquad (A10)$$

Eq. A10 may be rewritten to define the dilution rate as :

$$D = \mu - b \qquad (A11)$$

showing that the growth rate must be faster than the dilution rate by the amount of the decay rate. Substituting μ in Eq. A9 with Eq. A10 gives Eq. A12:

$$X_B = Y\ (S_{so} - S_s)\ /\ (1 + b\tau) \qquad \text{(A12)}$$

The observed yield is the measured biomass formed per substrate removed taking decay into account and is defined by:

$$Y_{obs} = X / (S_{so} - S_s) \qquad \text{(A13)}$$

with **X** the measured **biomass concentration**. Assuming negligible biomass debris as part of X (influenced by τ), results in X being equal to $X_B$. Combining Eqs. A12 and A13 gives the correlation between Y and $Y_{obs}$:

$$Y_{obs} = Y / (1 + b\tau) \qquad \text{(A14)}$$

Eq. A4 may be rewritten for substrate determination and μ substituted with Eq. A10, giving:

$$S_s = \mu K_s / (\mu_m - \mu)$$
$$= [K_s (1/\tau + b)]\ /\ [\mu_m - (1/\tau + b)] \qquad \text{(A15)}$$

iii) On COD: Investigating the oxygen required for aerobic respiration, it can be said from basic stoichiometry that the electrons removed from the substrate must end up in either the electron acceptor or the biomass formed. With COD a measure of the flow of electrons, the substrate COD removed, equals the biomass formed in COD plus the oxygen used in COD (electron acceptor). Therefore, **RO, the mass rate of oxygen utilised**:

$$RO = F(S_{so} - S_s) - Y_{obs}.F\ (S_{so} - S_s)$$
$$= F(S_{so} - S_s)\ (1 - Y_{obs}) \qquad \text{(A16)}$$

# APPENDIX B : EQUILIBRIUM CHEMISTRY

## 1. Theoretical background

Equilibrium chemistry is associated with the degree of dissociation of the weak acid / bases. Dissociation in turn is dependent on the dissociation constants, the total species concentrations and the ionic strength of electrolyte (Stumm & Morgan 1981). The pH of a solution can be calculated by equilibrium calculations, using (i) mass balance equations (total species concentrations), (ii) equilibrium relationships (equilibrium constants), (iii) correction for ionic strength (activity coefficients) and (iv) a proton condition (mass balance on protons) or charge balance (electro neutrality) (Snoeyink & Jenkins 1980). The method for the development of these equilibrium equations is well described in literature and will not be dealt with here. A comprehensive review and development on the topic were done by Loewenthal *et al.* (1989), Moosbrugger *et al.* (1993a, 1993b and 1993d) and Moosbrugger *et al.* (1993).

The development of equations for the solution in Chapter II Section 1.2, is as follows:

i) Mass balance equations for total species concentration:

| | |
|---|---|
| $C_{TC} = [H_2CO_3^*] + [HCO_3^-] + [CO_3^{2-}]$ | (Total carbonate species concentration) |
| $C_{TA} = [HAc] + [Ac^-]$ | (Total acetic acid species concentration) |
| $C_{TN} = [NH_4^+] + [NH_3]$ | (Total nitrogen species concentration) |
| $C_{TP} = [H_3PO_4] + [H_2PO_4^-] + [HPO_4^{2-}] + [PO_4^{3-}]$ | (Total phosphorus species concentration) |
| $C_{TNa} = [Na^+]$ (strong base) | (Total sodium concentration) |

where: [ ] molar mass concentration, mol/*l*

$[H_2CO_3^*]$ the sum of dissolved carbon dioxide and carbonic acid = $[CO_2]_{aq} + [H_2CO_3]$ (Stumm & Morgan 1970)

ii) Equilibrium relationships or dissociation equations:

| | | | |
|---|---|---|---|
| Water species: | $(H^+)(OH^-)$ | = | $K_W$ |
| Carbonate species: | $(H^+)(HCO_3^-) / (H_2CO_3^*)$ | = | $K_{C1}$ |
| | $(H^+)(CO_3^{2-}) / (HCO_3^-)$ | = | $K_{C2}$ |
| Acetic acid species: | $(H^+)(Ac^-) / (HAc)$ | = | $K_A$ |

| | | | |
|---|---|---|---|
| Nitrogen species: | $(H^+)(NH_3)$ / $(NH_4^+)$ | = | $K_N$ |
| Phosphorus species: | $(H^+)(H_2PO_4^-)$ / $(H_3PO_4)$ | = | $K_{P1}$ |
| | $(H^+)(HPO_4^{2-})$ / $(H_2PO_4^-)$ | = | $K_{P2}$ |
| | $(H^+)(PO_4^{3-})$ / $(HPO_4^{2-})$ | = | $K_{P3}$ |

where: ( ) activity (active mass) concentration mol/*l*

$K_x$ thermodynamic dissociation equilibrium constants, refer Table B1

$K_w$ thermodynamic ion product constant, refer Table B1

The dissociation and ion product constants are temperature dependent and defined in Table B1 below (Benefield *et al.* 1982; Loewenthal *et al*. 1989).

TABLE B1 - Equilibrium constants (T = °K)

| pK | Equation |
|---|---|
| $pK_w$ | 4787,3 / T + 7,1321 * log T + 0,010365 * T - 22,801 |
| $pK_{C1}$ | 3404,7 / T - 14,8435 + 0,03279 * T |
| $pK_{C2}$ | 2902,4 / T - 6,498 + 0,02379 * T |
| $pK_A$ | 1170,5 / T - 3,165 + 0,0134 * T |
| $pK_N$ | 2835,8 / T - 0,6322 + 0,00123 * T |
| $pK_{P1}$ | 799,3 / T - 4,5535 + 0,01349 * T |
| $pK_{P2}$ | 1979,5 / T - 5,3541 + 0,01984 * T |
| $pK_{P3}$ | 12,023 |

iii) Total species concentrations are determined analytically, giving mass concentration. To enable calculation with mass concentrations the dissociation equations are corrected with activity coefficients. The hydrogen ion concentration is however determined by a pH measurement, measuring activity, and is an exception and is used without a correction, giving :

$$pH = -\log (H^+)$$

$$(OH^-) = f_m [OH^-]$$

| | | | | | |
|---|---|---|---|---|---|
| water species: | $(H^+)\,[OH^-]$ | = | $K'_w$ | = | $K_w/f_m$ |
| carbonate species: | $(H^+)[HCO_3^-]\ /\ [H_2CO_3^*]$ | = | $K'_{C1}$ | = | $K_{C1}/f_m$ |
| | $(H^+)\,[CO_3^{2-}]\ /\ [HCO_3^-]$ | = | $K'_{C2}$ | = | $K_{C2}.f_m\ /\ f_d$ |
| acetic acid species: | $(H^+)\,[Ac^-]\ /\ [HAc]$ | = | $K'_A$ | = | $K_A/f_m$ |
| nitrogen species: | $(H^+)\,[NH_3]\ /\ [NH_4^+]$ | = | $K'_N$ | = | $K_N/f_m$ |
| phosphorus species: | $(H^+)\,[H_2PO_4^-]\ /\ [H_3PO_4]$ | = | $K'_{P1}$ | = | $K_{P1}/f_m$ |
| | $(H^+)\,[HPO_4^{2-}]\ /\ [H_2PO_4^-]$ | = | $K'_{P2}$ | = | $K_{P2}.f_m\ /\ f_d$ |
| | $(H^+)\,[PO_4^{3-}]\ /\ [HPO_4^{2-}]$ | = | $K'_{P3}$ | = | $K_{P3}.f_d/f_t$ |

where: $f_m$, $f_d$ and $f_t$, monovalent, divalent and trivalent activity coefficients, refer Table B2

$K'_x$ apparent dissociation equilibrium constants, refer Table B2

$K'_w$ apparent ion product constant, refer Table B2

The activity coefficients may be calculated using the Davies equation for solutions with ionic strength of less than 0,5 M (Stumm & Morgan 1981):

$$\log f_i = -Az_i^2\ [I^{½} / (1 + I^{½})\ -\ 0{,}3\ I] \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{ Davies Equation}$$

where: $f_i$ activity coefficient for ionic species i, giving $f_m$ , $f_d$ and $f_t$

$A = 1{,}825 \times 10^6\ (\varepsilon T)^{-3/2}$

$\varepsilon$ dielectric constant = 78,3

T temperature in Kelvin

$z_i$ charge of the $i^{th}$ species - mono = 1; di = 2 and tri = 3

I the ionic strength = $½ \Sigma\, c_i z^2_i$

$c_i$ concentration of the $i^{th}$ ionic species, mol/l (dissociated species)

Activity coefficients and equilibrium constants were calculated for an ionic strength of 0,1 M at a temperature of 25°C and shown in Table B2.

TABLE B2 - Apparent equilibrium constants corrected for ionic strength of 0,1 M at 25°C

| pK | Value |
|---|---|
| $pK'_w$ | 13,891 |
| $pK'_{C1}$ | 6,245 |
| $pK'_{C2}$ | 10,008 |
| $pK'_A$ | 4,648 |
| $pK'_N$ | 9,143 |
| $pK'_{P1}$ | 2,041 |
| $pK'_{P2}$ | 6,878 |
| $pK'_{P3}$ | 11,485 |
| $f_m = 0,780$ | $f_d = 0,371$ $f_t = 0,107$ |

iv) Proton Condition

The proton mass balance is established with reference to a reference level of protons. The reference level is taken as the species with which the solution was prepared. The species having protons in excess of the reference level are equated with the species having less protons than the reference level. This may be set out as in Fig. B1 resulting in the proton balance below :

$$[Na^+] + [H^+] = [HCO_3^-] + 2[CO_3^{2-}] + [Ac^-] + [NH_3] + [H_2PO_4^-] + 2[HPO_4^{2-}] + 3[PO_4^{3-}] + [OH^-]$$

There are 14 unknown species and 14 equations to solve the solution species concentrations. The total species concentrations $C_{TA}$, $C_{TN}$, $C_{TP}$ and $C_{TNa}$ are known from preparation of the feed solution or are analytically determined. The total carbonate species, $C_{TC}$, may be determined from the carbonate alkalinity and pH measurement (WRC 1986) or as in this case, for an open system, it is a function of $CO_2$ partial pressure.

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Species formed by | | | | | | | | | $Na^+$ | | $H^+$ | |
| gain of one proton: | | | | | | | | | ↑ | | ↑ | |
| **Reference species:** | **$H_2CO_3$*** | **---** | **HAc** | **---** | **$NH_4^+$** | **---** | **$H_3PO_4$** | **---** | **NaOH** | **---** | **$H_2O$** | |
| | ↓ | | ↓ | | ↓ | | ↓ | | | | ↓ | |
| Species formed by loss of one proton: | $HCO_3^-$ | | $Ac^-$ | | $NH_3$ | | $H_2PO_4^-$ | | | | $OH^-$ | |
| Species formed by loss of two protons: | ↓ $CO_3^{2-}$ | | | | | | ↓ $HPO_4^{2-}$ | | | | | |
| Species formed by loss of three protons: | | | | | | | ↓ $PO_4^{3-}$ | | | | | |

FIG. B1 - Proton balance

Using Henry's law constant, $K_H$, the dissolved $CO_2$ species may be calculated. The ratio of dissolved $CO_2$ to $H_2CO_3$ is fixed and equal to 99,76 : 0,24 at 25°C and is independent of pH and ionic strength (Stumm & Morgan 1970). The $H_2CO_3$* concentration may be approximated by the dissolved $CO_2$ concentration :

$$K_H \rho_{co2} = [CO_2]_{aq} \simeq [H_2CO_3^*]$$

with: $pK_H = -1760/T + 9,619 - 0,00753T$

$\rho_{co2}$ partial pressure of $CO_2$. The University of Pretoria is at an elevation of 1400 m above sea level with atmospheric pressure of approximately 85,5 kPa giving a partial pressure for $CO_2 \simeq$ 0,00027 atmosphere.

These equations can now be solved simultaneously to yield the concentration of each chemical species.

## 2. Experimental

### Computer Programme

The equations as developed above for an aerated solution with acetic acid, ammonium chloride, phosphoric acid and sodium hydroxide in distilled water were programmed in the spreadsheet program Excel(1998) for MSOffice. The pH was calculated for solutions with different total species concentrations by using the solver function, and compared to measured values of solutions prepared in a laboratory. Spreadsheet printouts of the programme are given below.

### Solution preparation and pH measurement

Solutions of different concentrations were made up in freshly distilled water adding ammonium chloride, phosphoric acid, acetic acid and sodium hydroxide which was aerated. The solution concentrations are summarised in Table B3 below.

The pH was measured for each solution with a Mettler MP120 pH meter and Mettler Inlab413 temperature compensating probe. The accuracy stated by the manufacturer is $\pm$ 0,01 pH units. Chemicals of AR quality were used. Measurement was carried out under careful constant and similar stirring conditions for all the solutions. pH calibration was done with pH buffers of 4,01 and 7,01 pH and tested against a 1,68 pH buffer. All glassware was thoroughly washed with hydrochloric acid (Standard Methods 1995).

### Results

The calculated and measured pH values are summarised in Table B3. The carbonate subsystem was only included in the calculation where indicated. A number of commercially available buffer solutions and self-prepared buffers were tested and compared. Big differences were noticed in some of them, notwithstanding guaranteed accuracies. The exercise emphasises the care that needs to be taken in using or selecting commercially available buffers for accurate calibration of pH meters.

TABLE B3 - Comparison of calculated and measured pH values

| Solution | Subsystem species added | Concentration mg/*l* | Temp °C | Measured pH | Calculated pH | pH Difference | Calculated I (mol/*l*) |
|---|---|---|---|---|---|---|---|
| 1a | $NH_4Cl$ – N | 449 | 24 | 5,23 | 5,33 (5,29)* | +0,10 (+0,06)* | 0,0357 |
| | | 125 | 24 | 5,43 | 5,65 (5,51) | +0,22 (+0,08) | 0,0089 |
| | | 50 | 24 | 5,49 | 5,86 (5,60) | +0,37 (+0,11) | 0,0036 |
| 1b | $H_3PO_4$-P | 620 | 25 | 2,09 | 2,04 | -0,05 | 0,0100 |
| | | 124 | 25 | 2,58 | 2,54 | -0,04 | 0,0020 |
| | | 62 | 25 | 2,82 | 2,79 | -0,03 | 0,0010 |
| 1c | HAc | 497 | 25 | 3,42 | 3,43 | +0,01 | 0,0002 |
| | | 99 | 27 | 3,80 | 3,79 | -0,01 | ~ 0 |
| | | 50 | 25 | 3,95 | 3,95 | 0 | ~ 0 |
| 2 | P/N/HAc | 50/50/50 | 23 | 2,89 | 2,86 | -0,03 | 0,0044 |
| | +NaOH | 49/49/49+63,5 | 24 | 4,06 | 4,02 | -0,04 | 0,005 |
| | +NaOH | 48/49/48+91,2 | 25 | 5,52 | 5,50 | -0,02 | 0,0057 |
| 3 | P/N/HAc | 50/100/99 | 26 | 2,93 | 2,86 | -0,07 | 0,0080 |
| | +NaOH | 49/98/97+63,5 | 26 | 3,89 | 3,84 | -0,05 | 0,0087 |
| | +NaOH | 48/97/96+118,5 | 26 | 5,47 | 5,52 | +0,05 | 0,0098 |
| 4 | P/N/HAc | 50/100/497 | 24 | 2,85 | 2,84 | -0,01 | 0,0080 |
| | +NaOH | 49/99/494+119 | 24 | 4,04 | 4,05 | +0,01 | 0,0101 |
| | +NaOH | 49/98/488+352 | 24 | 5,52 | 5,58 | +0,06 | 0,0158 |
| | +aerated (24h) | 49/98/488/352 | 18 | 5,51 | 5,58 | +0,07 | 0,0158 |

* Values in brackets includes the carbonate subsystem for an open system.

The differences between the calculated and measured pH values for the pure solutions were less than 0,1 pH units except for the $NH_4Cl$ solutions. The reason for the bigger differences for these solutions is not clear, but is probably related to the very low buffer capacity of the solutions in the measured pH range. It is however still relative accurate with differences of less than 0,4 pH units. The difference decreases as the nitrogen concentration increases and together with the negligible buffer capacity in the acidic range, makes the differences not important for the purpose of this study. The mixed solution differences were less than 0,1 pH units, indication accurate modelling by the calculation method. The concentrations of all the different species of each solution are not shown but are known through the calculation method. The solutions are therefore completely characterised.

Comparing the pH values for the different solutions, it is seen that the pH values are different and decreases with increase in concentration. The increased N and HAc concentrations for solution 3 versus 2, decreased the pH for the same NaOH dose. A similar result may be noticed for an increased HAc concentration for solution 4 versus 3. These results are expected considering equilibrium chemistry and the shift in the equivalence point with increased reference species.

The added strong base (NaOH) increased the pH as would be expected. The carbonate subsystem had virtually no influence on the acidic pH of approximately 5,6 for solution 4, but will have an increased influence on an increased basic solution (Stumm & Morgan 1981).

**Conclusions**

The test work confirmed that the solution could completely be characterised by equilibrium chemistry. The programme gave accurate predictions and can be used to calculate the pH due to changes in chemical species concentrations. The most important aspect is the confirmation that the pH, the controlled parameter, is determined by the weak acid and base subsystems and strong acid and/or base added to the solution. The selected pH for the visualised chemo-pHauxostat will fix the total species and subsystem species concentrations for a given feed solution composition. It is thus possible to calculate and predict the species concentrations at the selected pH set point.

# 3. Computer program printouts

**Properties**

| | | | |
|---|---|---|---|
| Temperature | T-C | **28.6** | oC |
| Ionic strength of solution feed | TDS feed | **920** | mg/l |
| Partial pressure of CO2 in atmosphere | Pco2 | **0.00027** | atm |
| Ionic strength of solution effluent | TDSe effluent | **640** | mg/l |
| pH of solution (initial) | pHi | 3.88 | |
| pH of solution (final) | pHf | 5.52 | |

**Activity coefficients**

| | | | | |
|---|---|---|---|---|
| Temperature | T | 301.6 | K | Effluent |
| Ionic strength of solution feed | I | 0.023 | Ie | 0.016 |
| Monovalent ions | fm | 0.8654584 | fme | 0.882969 |
| Divanlent ions | fd | 0.5610283 | fde | 0.6078297 |
| Trivalent ions | ft | 0.2724054 | fte | 0.326219 |
| Dielectric constant for water | D | 78.3 | ?? | |
| Henry's constant for [H2CO3*] | Kh | 0.0307321 | ?? | |
| | pKh | 1.5124082 | | |

**Molar Mass**

| | | | |
|---|---|---|---|
| H2PO4- | MM1 | 96985.8 | mg/mol |
| HPO42- | MM2 | 95977.9 | mg/mol |
| PO43- | MM3 | 94970 | mg/mol |
| CH3COO- | MM4 | 59043.7 | mg/mol |
| NH4+ | MM5 | 18038.6 | mg/mol |
| Na+ | MM6 | 22990 | mg/mol |
| P | MM7 | 30974 | mg/mol |
| N | MM8 | 14007 | mg/mol |
| Hac | MM9 | 60051.6 | mg/mol |
| NaOH | MM10 | 39996.9 | mg/mol |
| HCl | MM11 | 36460.9 | mg/mol |
| CH3CH2COOH | MM12 | 74078.4 | mg/mol |
| CH3CH2CH2COOH | MM13 | 88105.2 | mg/mol |
| COD/Hac (g/mol) | | 63.996 | |

**Concentrations**

| | | | | |
|---|---|---|---|---|
| | | | | I = pH |
| Phosphate Subsystem initial **P** | Ptmi | **51** | mg/l | 0.0230407 |
| Acetic Subsystem initial **Hac** | Atmi | **5000** | mg/l | |
| Ammon. Subsystem initial **N** | Ntmi | **146** | mg/l | I = pHi |
| Caustic dose **NaOH** | NaOHmi | 501.5 | mg/l | 0.0230407 |
| Caustic dose **NaOH** | NaOHmf | 501.5 | mg/l | |
| Propionic **Hpr** | Prtmi | **0** | mg/l | |
| Butryric **Hbu** | Btmi | **0** | mg/l | |
| | | | | I = pHf |
| Phosphate Subsystem final **P** | Ptmf | **16** | mg/l | 0.015985 |
| Acetic Subsystem final **Hac** | Atmf | **287** | mg/l | |
| Ammon. Subsystem final **N** | Ntmf | **52** | mg/l | |
| Propionic **Hpr** | Prtmf | **0** | mg/l | |
| Butryric **Hbu** | Btmf | **0** | mg/l | |
| Phosphate Subsystem initial | Pti | 0.0016465 | mol/l | |
| Acetic Subsystem initial | Ati | 0.0832617 | mol/l | |
| Ammonium Subsystem initial | Nti | 0.0104234 | mol/l | |
| Caustic dose NaOH | NaOHi | 0.0125385 | mol/l | |
| Caustic dose NaOH | NaOHf | 0.0125385 | mol/l | |
| Phosphate Subsystem final | Ptf | 0.0005166 | mol/l | |
| Acetic Subsystem final | Atf | 0.0047792 | mol/l | |
| Ammonium Subsystem final | Ntf | 0.0037124 | mol/l | |

**Dissociation constants' temperature dependency**

| | | | | |
|---|---|---|---|---|
| Water | KwT | 1.31327E-14 | pKwT | 13.881647 |
| Carbonate | Kc1T | 4.6264E-07 | pKc1T | 6.3347571 |
| | Kc2T | 5.00719E-11 | pKc2T | 10.300406 |
| Phosphate | Kp1T | 0.006834662 | pKp1T | 2.1652829 |
| | Kp2T | 6.4125E-08 | pKp2T | 7.1929729 |
| | Kp3T | 9.48418E-13 | pKp3T | 12.023 |
| Acetate | KaT | 1.7482E-05 | pKaT | 4.7574082 |
| Ammonium | KnT | 7.22291E-10 | pKnT | 9.1412879 |

**Activity corrections**

INFLUENT :

| | | | | |
|---|---|---|---|---|
| Water | Kw | 1.51742E-14 | pKw | 13.818893 |
| Carbonate | Kc1 | 5.3456E-07 | pKc1 | 6.2720033 |
| | Kc2 | 7.72423E-11 | pKc2 | 10.112145 |
| Phosphate | Kp1 | 0.007897159 | pKp1 | 2.1025291 |
| | Kp2 | 9.8921E-08 | pKp2 | 7.0047115 |
| | Kp3 | 1.9533E-12 | pKp3 | 11.709231 |
| Acetate | Ka | 2.01997E-05 | pKa | 4.6946544 |
| Ammonium | Kn | 8.34576E-10 | pKn | 9.0785341 |

EFFLUENT :

| | | | | |
|---|---|---|---|---|
| Water | Kwe | 1.48733E-14 | pKwe | 13.827592 |
| Carbonate | Kc1e | 5.23959E-07 | pKc1e | 6.2807026 |
| | Kc2e | 7.27373E-11 | pKc2e | 10.138243 |
| Phosphate | Kp1e | 0.007740546 | pKp1e | 2.1112284 |
| | Kp2e | 9.31517E-08 | pKp2e | 7.0308093 |
| | Kp3e | 1.76715E-12 | pKp3e | 11.752727 |
| Acetate | Kae | 1.97991E-05 | pKae | 4.7033536 |
| Ammonium | Kne | 8.18025E-10 | pKne | 9.0872334 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Calculatons** | | | pHi | **3.87939431232** | pHf | **5.52000000000** | |
| **Calculate initial equilibrium (proton balance) :** | | | | | | | |
| | | solver H= | Hs | 132009652.5 | | 3.879394312323 | pHs 3.8793943123 |
| NH3i | 6.58969E-08 | | H | 0.000132009652454 | pH | 3.879394312323 | |
| OHi | 1.14948E-10 | | **solve no CO2 :** | | **solve with CO2 :** | | |
| Aci | 0.01104968 | | | | | | |
| HCO3i | 3.36006E-08 | | **No Na+** | 12538438130.9572 | **No Na+** | 12538471732 | Wp 0.016716094 |
| CO3i | 1.96606E-14 | | | | | | Xp 0.000749347 |
| AlkH3PO4 | 0.001620704 | | **NaOHmi** | -33600.60204 | **NaOHmi** | 0 | Yp 1.47966E-08 |
| HPO4i | | | **NaOHmf** | -33600.60204 | **NaOHmf** | 0 | |
| PO4i | | | | | | | Wn 158175.702 |
| NaOHmi | **501.5** | mg/l | | | | | |
| NaOHmf | **501.5** | mg/l | | | | | |

**Solution : strong acid/base dose : initial and final known**

Verander pHi en pHf bo

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | pHi | 3.879394312 | pHf 5.52 |
| AlkiHac | 0.011049678 | | AlkfHac | 0.004146726 | Hi | 0.00013201 | Hf 3.01995E-06 |
| AlkiH3PO4 | 0.001620704 | | AlkfH3PO4 | 0.000531818 | Wai | 6.535217506 | Waf 0.15252943 |
| AlkiH2CO3* | 3.36006E-08 | | AlkfH2CO3* | 1.43971E-06 | Wni | 158175.702 | Wnf 3691.759254 |
| AlkiNH4 | 6.58969E-08 | | AlkfNH4 | 1.00533E-06 | Wpi | 0.016716094 | Wpf 0.000390147 |
| AlkiH2O | -0.000152531 | | AlkfH2O | -3.4153E-06 | Xpi | 0.000749347 | Xpf 0.030845417 |
| | | | | | Ypi | 1.47966E-08 | Ypf 5.85157E-07 |
| AlkiSol | 0.01251795 | | AlkfSol | 0.004677573 | | | |
| Delta AlkSol | -0.007840377 | | | | | | |
| NaOH dose (mg/l) | -313.6 | | HCl dose(mg/l) | 285.9 | | | |
| NaOHmi-NaOHmf | 0.0 | | | | | | |

**Solution : weak acid/base dose (Ac) : initial and final known FOR YIELD**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AlkiAc | -0.07221205 | | AlkfAc | -0.000632498 | -0.07157955 | | |
| AlkiH3PO4 | 0.001620704 | | AlkfH3PO4 | 0.000531818 | 0.001088886 | | |
| AlkiCO3 | -1.66289E-05 | | AlkfCO3 | -1.8035E-05 | 1.40604E-06 | | |
| AlkiNH4 | 6.58969E-08 | | AlkfNH4 | 1.00533E-06 | -9.3943E-07 | | |
| AlkiH2O | -0.000152531 | | AlkfH2O | -3.4153E-06 | -0.00014912 | | |
| AlkiSol | -0.07076044 | | Alk others | -0.00012112 | -0.07063932 | | |
| AlkfAc calcul | 0.07063932 | | | | SRT | 15.3 | |
| Atf calcul | -0.533759 | | | delta | HRT | 3.1 | |
| Atmf calcul | -32053.1 | 287.0 | Atmf | -32340.1 | X | 4.63 | |
| Atmi-Atmf calcul | -37053.1 | -4713 | Atmi-Atmf | 32340.1 | I | 64 | |
| | | | | | Yobs | 0.344 | |
| delta/Atmi-Atmf | 6.861883344 | 112.683 | delta/Atmf | | Yalk | 1.657805776 | |
| % | 686.19 | 11268.31 | % | | | | |

**Solution : weak acid/base dose (HAc) : initial and final known**

| | | | | |
|---|---|---|---|---|
| AlkiHAc | 0.011049678 | | AlkfHAc ? | |
| AlkiH2PO4 | -2.58386E-05 | | AlkfH2PO4 | 1.52555E-05 |
| AlkiH2CO3* | 3.36006E-08 | | AlkfH2CO3* | 1.43971E-06 |
| AlkiNH3 | -0.010423294 | | AlkfNH3 | -0.003711424 |
| AlkiH2O | -0.000152531 | | AlkfH2O | -3.4153E-06 |
| AlkiSol | 0.00044805 | | Alk others | -0.00369814 |
| AlkfHAc calcul | 0.004146 | | | |
| Atf calcul | 0.004779 | | | delta |
| Atmf calcul | 287.0 | 287.0 | Atmf | 0.0 |
| Atmi-Atmf calcul | -4713.0 | -4713 | Atmi-Atmf | 0.0 |
| delta/Atmi-Atmf | 7.83326E-06 | 0.000 | delta/Atmf | |
| % | 0.00 | 0.01 | % | |

# APPENDIX C : ALKALINITY

## 1. Defining alkalinity

Alkalinity is a measure against the equivalence point of an equivalent solution. Different alkalinities can be defined for different equivalent solutions depending on the reference species, with each alkalinity having its own equivalence point (Loewenthal *et al*. 1989). In terrestrial waters the carbonate subsystem normally dominates which resulted in the general practice to refer to carbonate alkalinity (alkalinity relative to the carbonic acid equivalence point) when mentioning Alkalinity. In effluents a number of other subsystems may however be present and may include the ammonia, phosphoric and SCFA subsystems as for the feed under discussion. The alkalinity of the feed is a solution alkalinity and is a combination of the different subsystem equivalent solutions, forming one combined equivalent solution with a solution equivalence point. The solution alkalinity is the proton accepting capacity of the solution relative to the solution equivalence point.

Loewenthal *et al.* (1991) defined the solution alkalinity as the sum of the alkalinities of the individual weak acids/bases relative to their respective selected reference species, plus the water subsystem alkalinity. The alkalinities for the different weak acid/base subsystems may be derived from a proton balance. Considering the conventional equation for Alkalinity:

$$\text{Alkalinity} = 2[CO_3^{2-}] + [HCO_3^-] + [OH^-] - [H^+]$$

which may be explained by completing a proton balance on a $H_2CO_3$* equivalent solution with addition of base BOH, depicted by:

$$\begin{array}{lccccc} & B^+ & & & & H^+ \\ & \uparrow & & & & \uparrow \\ \textbf{Reference species:} & \textbf{BOH} & \textbf{---} & \textbf{H}_2\textbf{CO}_3^* & \textbf{---} & \textbf{H}_2\textbf{O} \\ & & & \downarrow & & \downarrow \\ & & & HCO_3^- & & OH^- \\ & & & \downarrow & & \\ & & & CO_3^{2-} & & \end{array}$$

FIG. C1 - Proton balance for Alkalinity

The proton accepting capacity (Alkalinity) of the solution is now equivalent to the amount of base added to the equivalent solution. This amount will be back titrated to the equivalence point during alkalinity determination. The base added is:

$$[B^+] = 2[CO_3^{2-}] + [HCO_3^-] + [OH^-] - [H^+]$$

giving the conventional equation for Alkalinity and demonstrating that it is, and may be defined as $H_2CO_3^*$ alkalinity. Alkalinities for individual weak acid/base subsystems may similarly be derived and defined, giving:

| | | |
|---|---|---|
| HAc alkalinity | = | $[Ac^-] + [OH^-] - [H^+]$ |
| $H_3PO_4$ alkalinity | = | $3[PO_4^{3-}] + 2[HPO_4^{2-}] + [H_2PO_4^-] + [OH^-] - [H^+]$ |
| $NH_4^+$ alkalinity | = | $[NH_3] + [OH^-] - [H^+]$ |
| $H_2CO_3^*$ alkalinity | = | $2[CO_3^{2-}] + [HCO_3^-] + [OH^-] - [H^+]$ |

Considering these alkalinities, each alkalinity can be expressed as the sum of two alkalinities, associated with its reference species. Referring to Fig. C1, the two subsystem reference species in this case are $H_2CO_3^*$ and $H_2O$. These individual subsystem alkalinities were defined and expressed by Loewenthal and co-workers (1991) as "Alk (reference species)" giving:

$$H_2CO_3^* \text{ alkalinity} = \text{Alk } H_2CO_3^* + \text{Alk } H_2O$$
$$= 2[CO_3^{2-}] + [HCO_3^-] + [OH^-] - [H^+]$$

with: Alk $H_2CO_3^*$ - alkalinity of the carbonate subsystem with reference species $H_2CO_3^*$ and equivalent to $2[CO_3^{2-}] + [HCO_3^-]$

Alk $H_2O$ - alkalinity of the water subsystem with reference species $H_2O$ and equivalent to $[OH^-] - [H^+]$

giving the general equation:

$$\text{Solution alkalinity} = \Sigma \text{Alk}_i + \text{Alk } H_2O$$

with: $Alk_i$ - the subsystem alkalinity for the $i^{th}$ weak acid / base subsystem relative to its selected reference species.

Note that the water subsystem Alk $H_2O$ is only added once. The solution alkalinity for the feed and the reactor can now be defined as:

$$\text{Solution alkalinity} = \text{Alk HAc} + \text{Alk } H_3PO_4 + \text{Alk } NH_4^+ + \text{Alk } H_2CO_3^* + \text{Alk } H_2O$$

with reference species: HAc, $H_3PO_4$, $NH_4^+$, $H_2CO_3^*$ and $H_2O$ respectively,

and :

$$\text{Alk HAc} = [Ac^-]$$

$$\text{Alk } H_3PO_4 = [H_2PO_4^-] + 2[HPO_4^{2-}] + 3[PO_4^{3-}]$$

$$\text{Alk } NH_4^+ = [NH_3]$$

$$\text{Alk } H_2CO_3^* = [HCO_3^-] + 2[CO_3^{2-}]$$

$$\text{Alk } H_2O = [OH^-] - [H^+]$$

The SCFA subsystem alkalinities may for simplicity be represented by the acetic acid subsystem alkalinity because the ionisation constants for the SCFA's, typically of concern (acetic, propionic, butyric and valeric), differs only slightly from that of acetic acid and with HAc concentration normally the highest. The SCFA concentration are converted to HAc concentration and then considered as HAc, giving:

$$\text{Alk SCFA} \simeq \text{Alk HAc} = [Ac^-]$$

It was concluded in Chapter II that equilibrium chemistry can be used to characterise the feed and the reactor solutions. All chemical species concentrations are thereby known and the solution alkalinity can be calculated using the above equations.

## 2. Calculating alkalinity

Equations for the total species concentrations, dissociation equations and subsystem alkalinities for the substrate were given in Chapter II and above. These equations may be combined as demonstrated by Loewenthal *et al*. (1991) to simplify alkalinity calculations. Developed equations are summarised below:

$$\text{Alk HAc} = C_{TA} / (1 + W)$$

$$\text{Alk } Ac^- = - C_{TA}.W / (1 + W)$$

$$\text{Alk } H_3PO_4 = C_{TP} . (1 + 2X + 3XY) / (1 + W + X + XY)$$

$Alk\ H_2PO_4^- = C_{TP} . (-W + X + 2XY) / (1 + W + X + XY)$

$Alk\ NH_3 = -C_{TN} . W / (1 + W)$

$Alk\ NH_4^+ = C_{TN} / (1 + W)$

$Alk\ H_2O = 10^{pH-pK'_w} - 10^{-pH} / f_m$

$Alk\ H_2CO_3^* = 2[CO^{2-}_3] + [HCO_3^-]$

$= K_H\rho_{CO2} [2(K'_1K'_2 (10^{pH})^2 + K'_1 10^{pH}]$

$Alk\ CO_3^{2-} = -2 [H_2CO_3^*] - [HCO_3^-]$

$= K_H\rho_{CO2} (-2 - K'_1 10^{pH})$

with: $W = 10^{pK'_1-pH}$

$X = 10^{pH-pK'_2}$

$Y = 10^{pH-pk'_3}$

$K'_1$ = first apparent dissociation equilibrium constant

$K'_2$ = second apparent dissociation equilibrium constant

$K'_3$ = third apparent dissociation equilibrium constant

# APPENDIX D : PHOTO PRINTS

## 1. Laboratory set-up

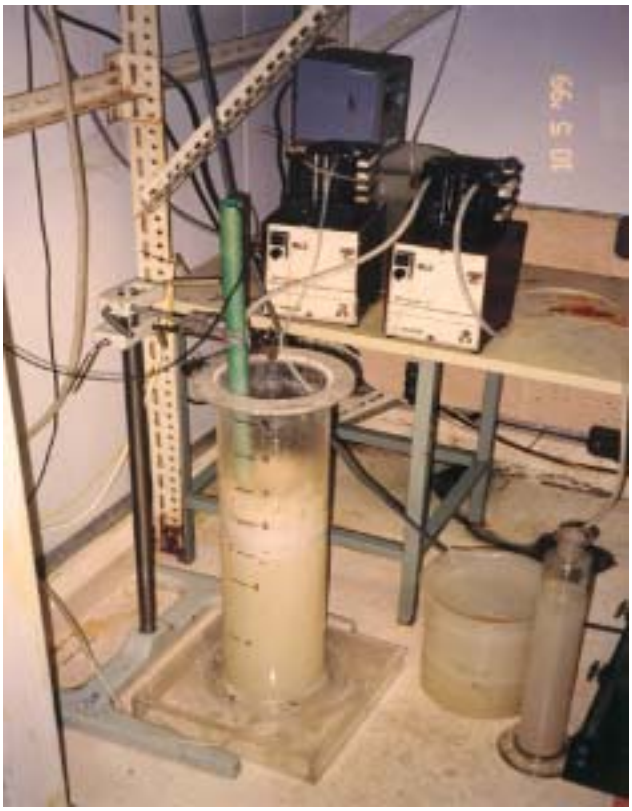

FIG. D1 - pHauxostat reactor Test Run A

FIG. D2 - pHauxostat reactor Test Run B

FIG. D3 - Top view

FIG. D4 - Side view

FIG. D5 - Sample points (bottom)

FIG. D6 - Air supply (bottom)