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Probing Control of Glucose Feeding in Cultivation of *Saccharomyces cerevisiae*

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Probing Control of Glucose Feeding in Cultivation of *Saccharomyces cervisiae*
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Abstra

In order to maximize the biomass yield in fed-batch cultivations of *Saccharomyces cerevisia*e, a pulse feeding strategy originally developed by Mats Åkesson at the department of automatic con-trol (Åkesson, 1999), was implemented. The controller was intended to keep the specific glucose uptake rate, $_{q}$s, below the critical specific glucose uptake rate $_{qs\ crit}$, to avoid over-flow metabolism. Simulations, made to see if the method worked, and to find optimal working conditions, were done before the experiments. A robust PID controller was developed in order to regulate the dis-solved oxygen tension at 30%, by changing the stirrer speed.
Two different commercial strains of yeast (from Jästbolaget AB, Sweden) were used: the so-called blue yeast, for ordinary doughs, and the so-called red yeast, for sweet doughs. Glucose was the carbon source in the cultivations. The specific glucose uptake rate, $_{q}$s, was controlled by the feed rate. If an up pulse in the feed rate resulted in a decrease of the DOT, below a certain point, the feed rate was increased in proportion to .DOT. If $_{qs}$ exceeded $_{qs\ crit}$ no decrease in DOT would be seen, and therefore the feed rate was decreased. When the stirrer speed was close to its maximum value, a safety net in the regulator prevented further feed rate increase.
With the implemented control strategy growth was fully respirative. This was shown by high bio-mass yield values, low glucose and ethanol concentrations during the fed-batch cultivations, and also by a RQ, close to 1.08, throughout the fed-batch experiments. In some of the fed batch ex-periments, indications of synchronization of the culture could be seen.

The µ values were slightly lower than expected. Therefore one may suspect that $_{qs}$ never reached $_{qs\ crit}$, before the feed rate was decreased due to saturation in the oxygen transfer. In order to im-prove the method, parameters like pulse length and initial cell mass concentration will have to be adjusted.

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Probing control of glucose feeding in cultivation of *Saccharomyces cerevisiae*

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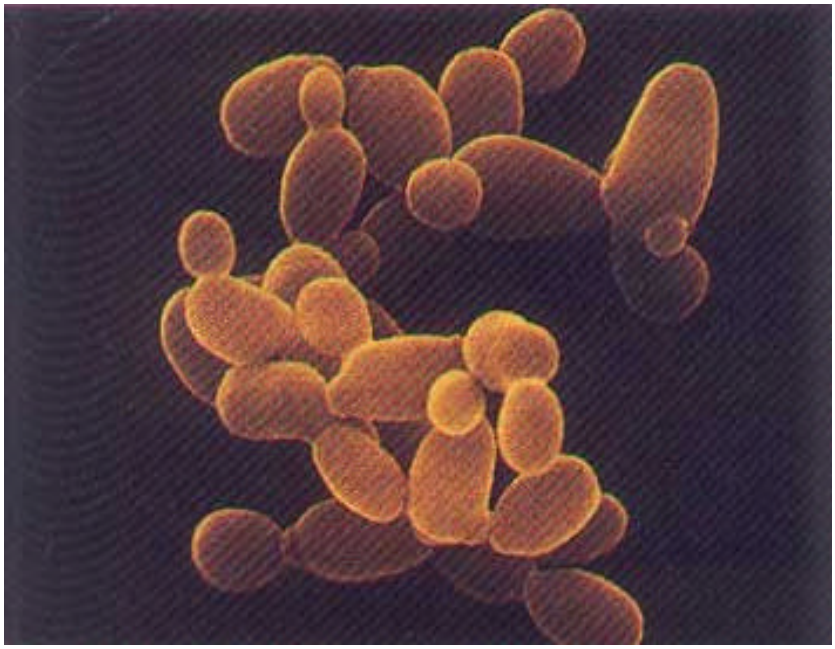

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Abstract

In order to maximize the biomass yield in fed-batch cultivations of *Saccharomyces cerevisiae*, a pulse feeding strategy originally developed by Mats Åkesson at the department of automatic control (Åkesson, 1999), was implemented. The controller was intended to keep the specific glucose uptake rate, q_s, below the critical specific glucose uptake rate q_s^{crit}, to avoid over-flow metabolism. Simulations, made to see if the method worked, and to find optimal working conditions, were done before the experiments. A robust PID controller was developed in order to regulate the dissolved oxygen tension at 30%, by changing the stirrer speed.

Two different commercial strains of yeast (from Jästbolaget AB, Sweden) were used: the so-called blue yeast, for ordinary doughs, and the so-called red yeast, for sweet doughs. Glucose was the carbon source in the cultivations. The specific glucose uptake rate, q_s, was controlled by the feed rate. If an up pulse in the feed rate resulted in a decrease of the DOT, below a certain point, the feed rate was increased in proportion to ?DOT. If q_s exceeded q_s^{crit} no decrease in DOT would be seen, and therefore the feed rate was decreased. When the stirrer speed was close to its maximum value, a safety net in the regulator prevented further feed rate increase.

With the implemented control strategy growth was fully respirative. This was shown by high biomass yield values, low glucose and ethanol concentrations during the fed-batch cultivations, and also by a RQ, close to 1.08, throughout the fed-batch experiments. In some of the fed batch experiments, indications of synchronization of the culture could be seen.
Obtained biomass yields on glucose were:

$Y_{x/s}$ (“Blue” strain)	0.46 ± 0.02 g/g
$Y_{x/s}$ (“Red” strain)	0.48 ± 0.01 g/g

μ (“Blue” strain)	0.10 - 0.12 h^{-1}
μ (“Red” strain)	0.08 - 0.11 h^{-1}

(obtained during the exponential feeding period)

The μ values were slightly lower than expected. Therefore one may suspect that q_s never reached q_s^{crit}, before the feed rate was decreased due to saturation in the oxygen transfer. In order to improve the method, parameters like pulse length and initial cell mass concentration will have to be adjusted.

Picture on the front page: (Microbiology, third edition, Prescott, Harley and Klein, 1996)

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1 Introduction

1.1 Objectives of study

Saccharomyces cerevisiae is an important industrial microorganism. The traditional processes in which it is used, beer and wine manufacturing, as well as Baker's yeast production, are large scale processes. Novel applications of *Saccharomyces cerevisiae* have been developed during later years. An example is the production of insulin for diabetics.

In these novel applications it is important to get a high biomass yield. This is our aim in the project, and the way to do this could be to implement a pulse feeding strategy originally developed for *Escherichia coli* by Mats Åkesson, Department of automatic control, LTH, Sweden (Åkesson, 1999). The strategy will help us to avoid over-flow metabolism, a phenomenon that leads to ethanol production and decreased biomass yield, even at aerobic growth.

The problem consisted of two parts: to develop and implement a pulse-feeding regulator, and to develop a robust and stable PID controller, which would control the dissolved oxygen concentration in a fast and reproducible way, for different conditions.

Initial work was done to describe the system with a simplified dynamic model that worked for the conditions that appeared. Through simulations we tested if our control strategy worked and determined optimal working conditions. Some test-fed-batch cultivations were made to determine K_La (Appendix J) and to tune parameters for the oxygen-controller. A number of fed-batch cultivations with pulse feeding were made. Two different commercial strains of *Saccharomyces cerevisiae*, obtained from Jästbolaget AB, Sweden, were used in order to test the robustness of the method. The success of the control was evaluated from measurements of μ, $Y_{x/s}$ and RQ.

The principal advantage of the method is its simplicity. No expensive or complicated analysis equipment is used, and the method can be used in different systems.

1.1 A little microbiology

All yeasts belong to the fungi family. They are not filamentous, like moulds, but are egg shaped, well-bordered cells. Their size varies but the cell diameter is usually around 10 µm, compared to around 1 µm for the well-known bacterium *Escherichia coli.*

Yeasts belong to the group of eukaryotic cells, i.e. they have a nuclear membrane. They contain organelles surrounded by plasma membranes e.g. mitochondria, the golgia apparatus and peroxisomes. The genome is situated in the nucleus, which is surrounded by a plasma membrane. The yeast cell is protected by a tough cell wall of chitin covered on the inside with a cell membrane. (Prescott, et al., 1996)

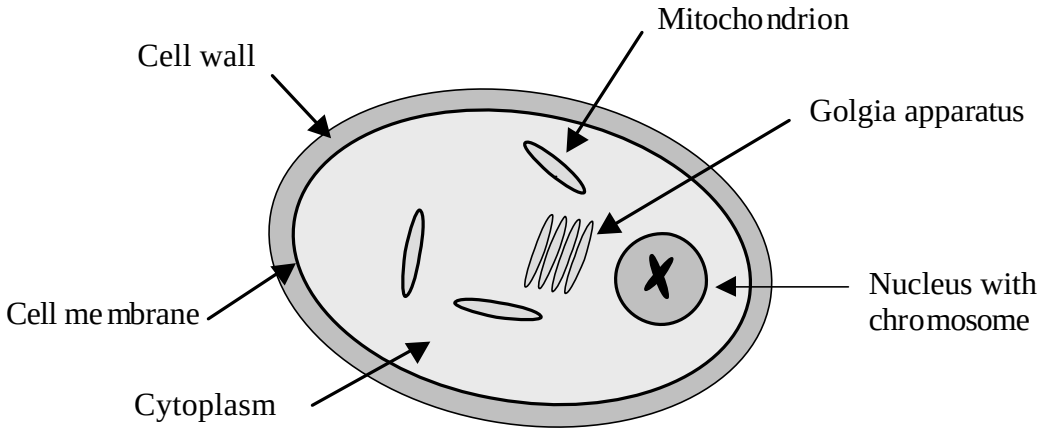

Figure 1.1 A simple sketch of a yeast cell.

During vegetative growth most yeast strains reproduce by budding, and not by fission like bacteria. After the budding process a scar is left on the surface of the mother cell. When scars cover the entire surface, the cell dies.

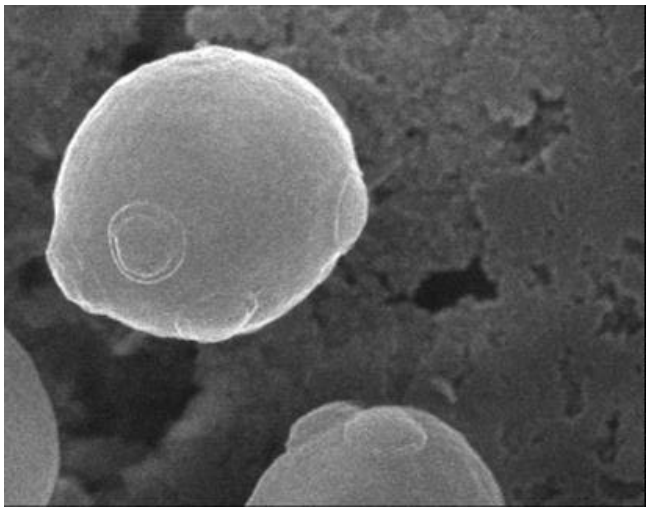

Figure 1.2 A magnified yeast cell. Notice the bud scars.

The yeast used in the present work, *Saccharomyces cerevisiae*, belongs to the group of glucose sensitive yeasts, which means that ethanol is formed in a fermentative process, when there is an excess of glucose (even in the presence of oxygen). Glucose insensitive yeasts like *Trichosporum cutaneum* degrade glucose exclusively in a respiratory way. (Fiechter, Seghezzi, 1992).

1.1.1 Areas of use

Saccharomyces cerevisiae is used as baker's yeast, for leavening of bread. This phenomenon is due to carbon dioxide formation in the absence of oxygen. The use of yeast in bread also affects the taste, by the formation of several esters.

- Some bulk proteins and enzymes that are abundant in *Saccharomyces cerevisiae* are derived on an industrial basis. One of these is invertase, which is used to convert sucrose to glucose and fructose (Hay, 1993)

- An important area of use for *Saccharomyces cerevisiae* is in the production of ethanol either as ingredients in different beverages or as fuel and disinfectant. Here the ability of the yeast to degrade different sugars to ethanol in the absence of oxygen is used.

- Because of the richness in proteins, minerals and vitamins, baker's yeast can be used in for example dietary supplements. Yeast extract is a well-known source of nutrients in cultivations of micro organisms (Hay, 1993).

- In later years *Saccharomyces cerevisiae* has been increasingly important in the production of recombinant proteins, e.g. insulin. Also vaccine against Hepatitis B is produced in *Saccharomyces c.* cultures.

In a batch cultivation, the entire carbon source is added before the batch start. For baker's yeast, a procedure like this results in over-flow metabolism and decreased biomass yield (see chapter 2).
Baker's yeast is normally produced using fed-batch cultivation. The advantage by using the fed-batch technique is, that it is possible to balance the addition of carbon source in such a way, that over-flow metabolism can be avoided.

Synthetic medium is mostly used on a lab-scale. The carbon source is often glucose, which gives opportunities to control the carbon flow in a more accurate way. A certain amount of different salts are added to fulfill the cell demand for compounds such as N, K, Mg, P, S and trace metals. It is important that the media contains enough of these salts; otherwise growth will decrease or cease even though the carbon source is not depleted.
An industrial (complex) medium is one with a not fully defined composition. It contains a carbon source and often N, K, Mg, P, S and trace metals as well, even if some compounds have to be added. Well-known examples of this are media based on molasses or yeast extract. These are preferable from an economical point of view, and especially media based on molasses are commonly used when baker's yeast is produced in a larger scale.

2 Biochemical background

If yeast is to be used in processes, where whole cells, or components of it are used, the biomass yield, $Y_{x/s}$, should be as high as possible.
To achieve a high cell yield, it is desirable to obtain fully respiratory growth on glucose.
However since *S. cerevisiae* is a Crabtree positive yeast, meaning that ethanol production and fermentative growth can occur even at aerobic conditions (if the glucose uptake is large enough), the feed rate has to be controlled carefully.

These regulatory problems originate from the fact that the maximum specific glucose uptake rate, q_s^{max}, in *Saccharomyces cerevisiae* does not match the maximum specific oxygen uptake rate, q_o^{max} (the maximum oxygen uptake is insufficient for complete oxidation of the maximum glucose uptake).
This means that the respiratory part of the catabolism actually can be a bottleneck at high glucose concentrations (Sonnleitner and Käppeli, 1986). The glucose uptake that corresponds to q_o^{max}, is called the critical glucose uptake rate, q_s^{crit} and is only about 1/7 of q_s^{max}.
When the glucose uptake rate exceeds q_s^{crit} the purely oxidative parts of the metabolism, the respiratory chain and to a certain degree also the TCA cycle are being saturated, and in order to regenerate the excess of NADH and $FADH_2$ produced in the glycolysis and the TCA-cycle, that can't be oxidized in the respiratory chain, some of the pyruvate produced in the glycolysis is reduced to ethanol. (figure 2.1 and 2.3). This process when some of the substrate in degraded without assistance from the respiratory chain is said to be respiro-fermentative.
The ATP / glucose yield in this process is lower than in a truly respirative process, (figure 2.3) explaining the lower cell mass yield on glucose.

In the absence oxygen the only way for the cell to produce essential ATP molecules is by so called substrate level phosphorylation (figure 2.3, example c). As mentioned above this process is said to be completely fermentative, resulting in a low growth rate.
The produced NADH and $FADH_2$ molecules can't be regenerated (oxidized) in the respiratory chain due to the absence of an electron acceptor (O_2), therefore pyruvate has to be reduced to ethanol.
Growth under anaerobic conditions partially inactivates the enzymes of the TCA-cycle and especially the respiratory chain.

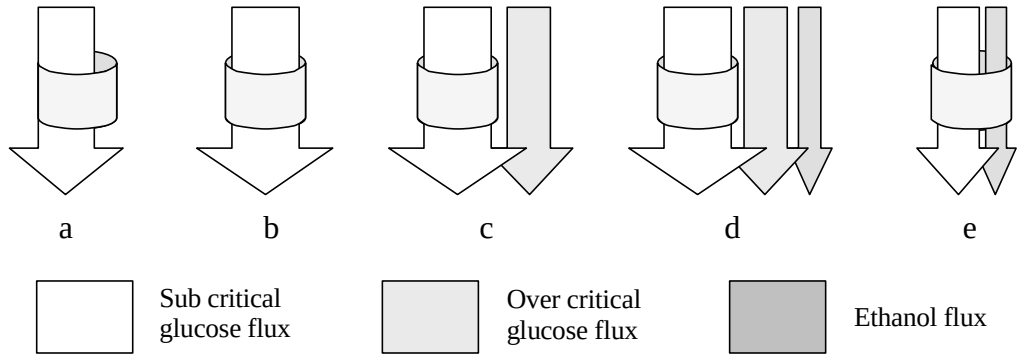

Figure 2.1 Different metabolic scenarios for a yeast cell: **a)** The glucose uptake is small enough and causes no over flow metabolism, so called sub critical flow. **b)** Critical flow, the glucose uptake matches the oxidative capacity. **c)** The glucose uptake is greater than the oxidative capacity, which results in ethanol production. **d)** Excess of glucose results in ethanol production and additional ethanol in the medium remains unused. **e)** The glucose flux is sub critical and if ethanol is available in the media it can be metabolised oxidatively.

Ethanol present in the media, either due to addition or over-flow metabolism can be metabolised by the yeast cells but only in the presence of oxygen (no substrate level phosphorylation is possible with ethanol as the only carbon source).
In other words; the ethanol degradation q_e is controlled by the oxygen uptake q_o. Glucose is the preferred substrate, but if the glucose concentration is low enough not to exceed the respirative capacity ethanol can be co-metabolised with glucose (figure 2.1, example e).

Ethanol is a more reduced compound than glucose; the degree of reduction is 6 for ethanol compared to 4 for glucose. Each c-mole of ethanol demands more oxidizing power than a c-mole of glucose, thus ethanol consumption leads to a larger production of NADH, which in turn leads to a higher O_2 consumption. This fact can be observed when we later look at the RQ-values in the cultivations (see Appendix J for an explanation of RQ).

In a batch phase these mentioned phenomena results in so called diauxic growth. Initially, the sugar is present in excess resulting in both respirative and fermentative break down of the substrate. When the primary substrate is used up the cells starts to break down the ethanol produced in the first phase. There are also at least two short periods of acetate degradation.

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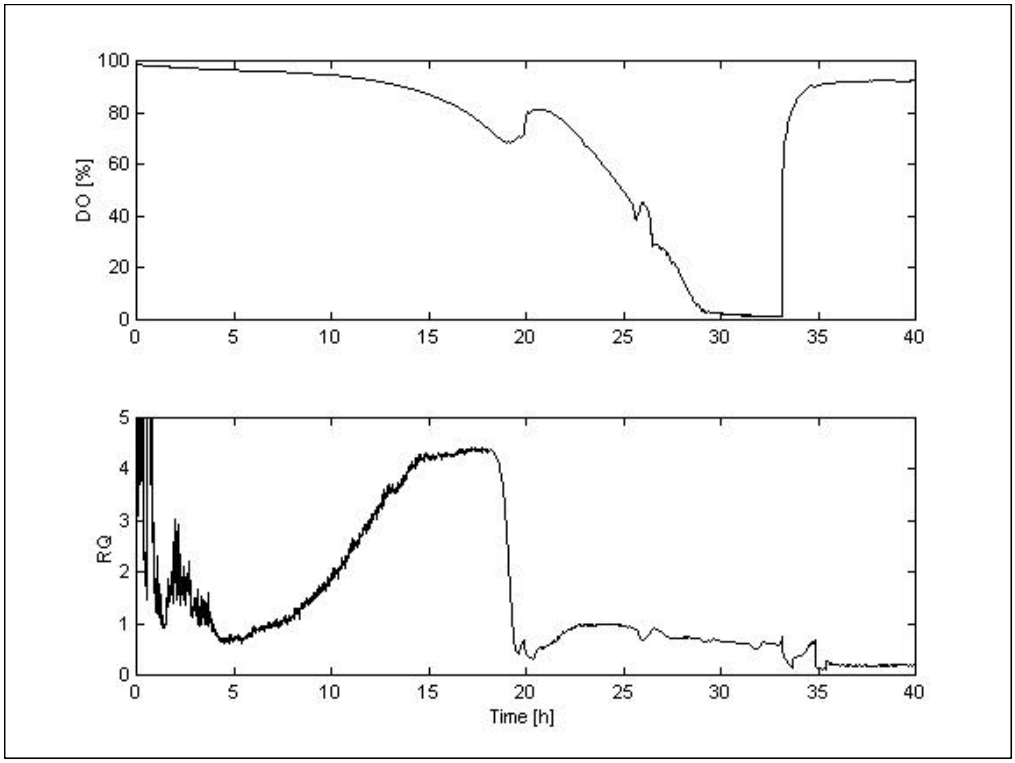

Figure 2.2 An example of diauxic growth of S. cerevisiae in a batch phase. **1)** In the first phase the glucose, which is present in excess, is degraded in a respiro-fermentative manner. When the glucose is used up the DOT temporarily rises. Soon the ethanol produced during the first phase is degraded leading to an additional decrease in DOT. When the ethanol has been depleted no further substrate remains, resulting in a sharply raised DOT-profile. **2)** Another way to study the diauxic growth of yeast in a batch phase is by looking at the RQ. An RQ-value higher than 1.1 as during the first 20 h indicates that the yeast grows in a respiro-fermentative way. RQ-values under 1.1 as in the end of the batch are due to ethanol consumption.

A sudden exposure to high glucose concentrations leads to over-flow metabolism for immediate kinetic conditions. However, there are also long-term regulatory effects caused by glucose, in which enzymes in the respiratory chain and the TCA-cycle are repressed due to high metabolite or glucose concentrations. This is a process known as catabolite repression.

Glucose is transported by many different uptake systems. Roughly the uptake systems can be grouped into high and low affinity systems. Low affinity systems will
normally not be quantitatively important at low glucose concentrations.

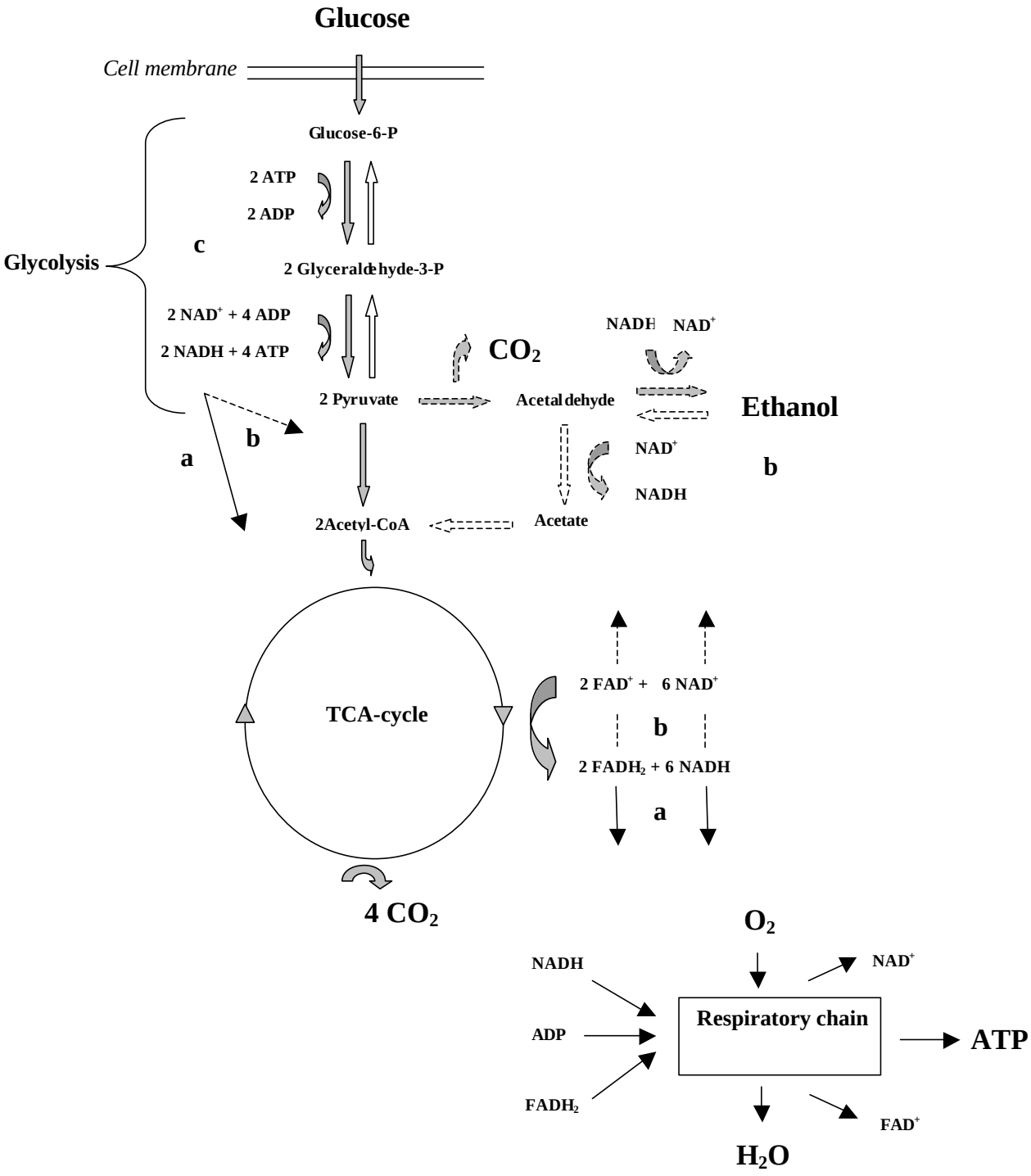

Figure 2.3 A schematic picture of the yeast catabolism. It must be remembered, that intermediates are withdrawn for biosynthesis. **a)** When the process is completely respirative all NADH and $FADH_2$ formed, are oxidized in the respiratory chain. **b)** When the glycolytic flux exceeds the respiratory capacity, either due to lack of oxygen or because $q_s > q_s^{crit}$, some of the NADH and $FADH_2$ produced are regenerated when pyruvate is reduced to ethanol. **c)** When no oxygen is available no NADH or $FADH_2$ molecules can be oxidized in the respiratory chain. All of them are regenerated in process b. The only ATP molecules formed are those produced in the glycolysis by so-called substrate level phosphorylation.

3 Reactor model

A model, which describes the behavior of the system and its response to changes in the feed rate, is useful when describing the dynamics of the system. The model is used for calculations of controller signals, and to simulate the system.
For this work, a simple model, that could give a realistic response to short term changes of the feed rate, was desired. To obtain such a model, we must have some understanding of what happens to the cells at molecular level. The model used is based on the model in Åkesson, 1999. It can be shown that the basic mass balance equations are:

$$\frac{dV}{dt} = F$$
$$\frac{d(VX)}{dt} = \mu \cdot VX$$
$$\frac{d(VS)}{dt} = FS_{in} - q_s \cdot VX$$
$$\frac{d(VE)}{dt} = (q_e^p - q_e^c) \cdot VX$$
$$\frac{d(VC_o)}{dt} = K_L a(N) \cdot V(C_o^* - C_o) - q_o \cdot VX$$

Eq 3.1

Where F is the feed rate of the glucose solution (l/h),
V is the total liquid volume in the reactor (l),
X is the biomass concentration (g/l),
μ is the specific growth rate (h^{-1}),
S is the substrate (glucose) concentration (g/l),
C_o is the dissolved oxygen concentration (g/l),
E is the ethanol concentration (g/l).
K_La is a measurement of the oxygen transfer in the reactor (h^{-1})
C_o^* is the maximal possible dissolved oxygen concentration (g/l)

Some of the parameters used in the model are not explained in this chapter. A short explanation of these is given in Appendix J.

The oxygen sensor can be approximated as a first order system where the time constant is possible to determine experimentally. This time constant should not change during the cultivations. O is the dissolved oxygen tension given as a percentage ratio of its maximum value. O_p is the measured dissolved oxygen tension.

$$\frac{dO_p}{dt} = \frac{1}{T_p} O - \frac{1}{T_p} \cdot O_p$$

Eq 3.2

The glucose uptake is assumed to follow Monod kinetics, where q_s^{max} is the maximum specific glucose uptake rate possible for *Saccharomyces cerevisiae*. K_s is the saturation parameter for glucose uptake.

$$q_s(S) = q_s^{\max} \cdot \frac{S}{K_s + S}$$

Eq 3.3

The maintenance glucose uptake q_{mc} is a part of the total q_s, that is not used for cell growth, but for life sustaining processes. When q_s is below q_{mc}, the entire glucose uptake is used for maintenance.

$$q_m = \min(q_s, q_{mc})$$

Eq 3.4

The amount of glucose, which is taken up by the cells and not used for cell maintenance, is used for further cell growth.

$$q_s^g = q_s - q_m$$

Eq 3.5

The total substrate uptake rate can be split into two components, one part used for oxidative growth, where oxygen is consumed, and one part used for fermentative uptake growth without oxygen consumption.
From the expression of the specific oxidative growth uptake of glucose, it can be seen that the specific oxygen uptake also follows Monod kinetics. K_o is called the saturation parameter for oxygen uptake.

Oxidative growth:

$$q_s^{g,ox} = \min\left(\frac{q_o^{\max} \cdot \frac{C_o}{K_o + C_o} - q_m Y_{o/s}^m}{Y_{o/s}^{ox}}, q_s^g \right)$$

Eq 3.6

At high glucose concentrations, the uptake of glucose exceeds by far the capacity of complete oxidation. The growth will partly be fermentative even though respiratory conditions are maintained.

Fermentative growth:

$$q_s^{g,fe} = q_s^g - q_s^{g,ox}$$

Eq 3.7

The resulting ethanol production can be described by the following equation.

Ethanol production:

$$q_e^p = q_s^{g,fe} \cdot Y_{e/s}$$

Eq 3.8

Ethanol consumption follows Monod kinetics. In this model, no glucose inhibition is included. The actual consumption of ethanol, called q_e^c, is limited by the free respiratory capacity.

$$q_e^{c,pot} = q_e^{c,\max} \cdot \frac{E}{K_e + E}$$

Eq 3.9

$$q_e^c = \min\left(\frac{q_o^{\max} \cdot \frac{C_o}{k_o + C_o} - q_s^{g,ox} \cdot Y_{o/s}^{ox} - q_m Y_{o/s}^m}{Y_{o/e}}, q_e^{c,pot} \right)$$

Eq 3.10

The total cell growth can be written as a sum of three terms, respirative glucose growth, fermentative glucose growth and growth on ethanol. Growth on ethanol and fermentative glucose growth will never occur at the same time, since ethanol is either produced or consumed.

Specific growth rate:

$$m = q_s^{g,ox} \cdot Y_{x/s}^{ox} + q_s^{g,fe} \cdot Y_{x/s} + q_e^c Y_{x/e}$$

Eq 3.11

Oxygen is used for oxidative growth, maintenance and ethanol consumption.

Specific oxygen uptake rate:

$$q_o = q_s^{g,ox} \cdot Y_{o/s}^{ox} + q_m Y_{o/s}^m + q_e^c Y_{o/e}$$

Eq 3.12

According to the model, the specific oxygen uptake will be saturated when the specific glucose uptake rate is equal to the critical specific glucose uptake rate.

$$q_o^{\max} = \left(q_s^{crit} - q_{mc}\right) Y_{o/s} + q_m Y_{o/s}^m$$

Eq 3.13

It is possible to calculate q_s^{crit} form this expression.

3.1 Linearization of the system

Since X and V do not change very rapidly, they can be considered to be constant. It has been shown in (Åkesson, 1999), that the system can be approximated by

$$T_s \frac{d\Delta q_s}{dt} + \Delta q_s = K_s \Delta F$$
$$T_o \frac{d\Delta O}{dt} + \Delta O = K_o \Delta q_s$$

Eq 3.1

The time constants and gains are given by

$$T_s = \left(\frac{\partial q_s}{\partial S} X \right)^{-1}$$
$$T_o = \left(K_L a \right)^{-1}$$
$$K_s = \frac{S_{in}}{VX}$$
$$K_o = -Y_{os} HX \left(K_L a \right)^{-1}$$

Eq 3.2

Note that K_s and K_o are gains, and not saturation parameters, in this context.

During long experiments these parameters change significantly. The system responds much faster when the cell mass and stirrer speed has increased. Together with the model for the oxygen sensor, the whole system, from glucose feed rate to dissolved oxygen measurements, these equations give a third order system with a potential time delay.
The overall stationary gain has been shown to be

$$K = K_s K_o = -\frac{\left(O^* - O_{sp} \right)}{\left(1 + a \right) F}$$

Eq 3.3

a is close to zero for low ethanol concentrations but may approach one for low glucose uptake rates or high ethanol concentrations. It may be larger than one for some conditions but normally it is between 0 and 1.

3.2 Parameter determinations

To be able to make realistic simulations of the system, the oxygen transfer coefficient, K_La, and the time constant of the oxygen sensor, T_p, must be determined.

3.2.1 Determination of K_La

K_La-values were determined for different stirrer speeds at two conditions selected to represent the beginning and the end of a typical fed-batch experiment. Two successful tests were made:

1) The medium volume was 1.4 l and the cell density 12 g/l.
2) The medium volume was 2.0 l and the cell density 60 g/l

Before the tests were made 6 drops of Antifoam 289 were added. The temperature of the media (The same composition as in the batch cultivations, chapter 6.2.1) was held at 30° C and pH was adjusted to 5.0 by addition of 2M NaOH. The stirrer speed was set to 550 rpm and by controlling the feeding of 0.4 kg/l glucose solution DO was stabilized around 30 %. After ca 10 min the stirrer speed was raised to 600 rpm while the feed rate remained constant. This procedure was then repeated by intervals of 100 rpm up to 1200 rpm. The gas analyzer was connected to give the amount of oxygen present in the out gas (for equipment specifications see chapter 6.1). The calculations are explained in Appendix G.

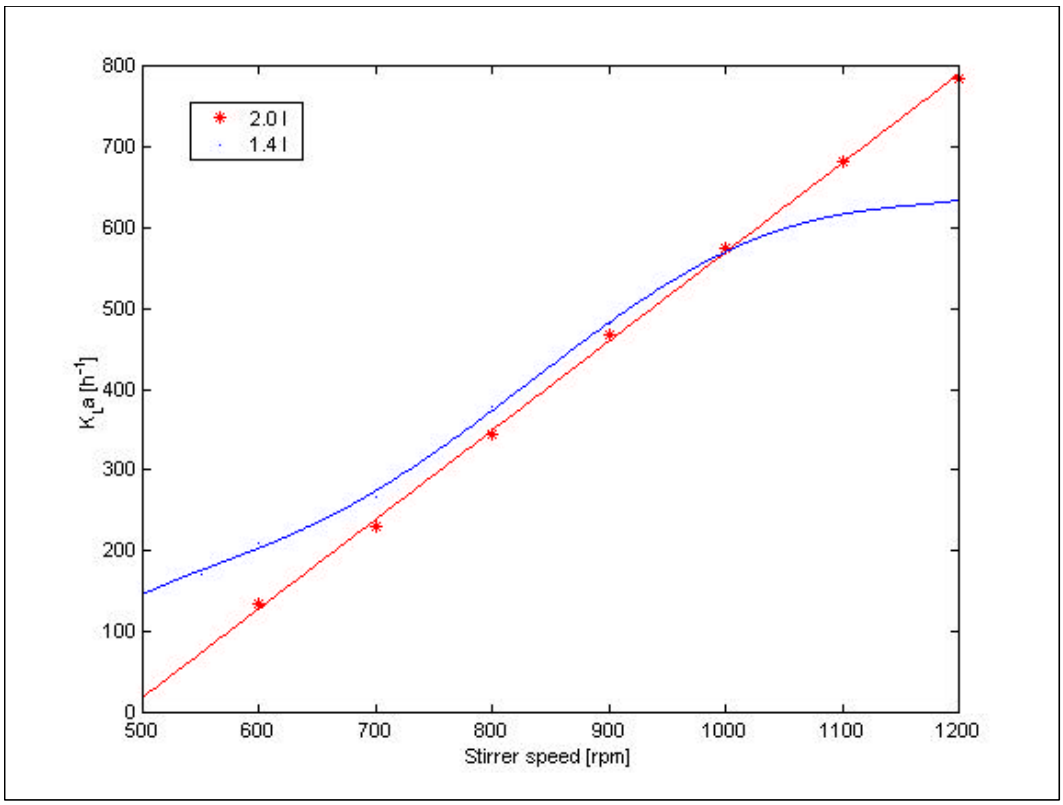

Figure 3.1 K_La characteristics for two different media volumes.

3.3 Linearization of the oxygen transfer function.

During a fedbatch experiment, the stirrer speed will always be between 550-1200 rpm, and thus a linear relation with respect to the stirrer speed can be used for linearization at higher volumes.

$$K_L a(N) = a \cdot (N - N_0)$$

Eq 3.1

a = 1.1 $h^{-1}rpm^{-1}$
N_0 = 483 rpm

for a volume of 2.0l.

For lower volumes, the partial derivative with respect to the stirrer speed will vary significantly, and thus it cannot be approximated by a constant. A polynomial of order five was fitted to the measurement points, and the partial derivative was calculated from this polynomial.
The expression of the fifth-order polynomial is not included here. It is very sensitive to variations in the coefficients and many decimals have to be included. At 800 rpm, the slope is 1.1 $h^{-1}rpm^{-1}$ and at 1100 rpm, the slope is 0.2 $h^{-1}rpm^{-1}$.

The oxygen sensor in the reactor does not measure the dissolved oxygen concentration, but the *dissolved oxygen tension*, which is the partial pressure of oxygen in equilibrium with the medium in the reactor. 100% equals an oxygen-saturated solution (Enfors and Häggström, 1998).

The mass balance equation for dissolved oxygen in the reactor can be described as

$$\frac{dO}{dt} = K_L a(N) \cdot (O^* - O) - q_o \cdot HX = f(N, O, X)$$

Eq 3.2

O^* is the dissolved oxygen tension for an oxygen saturated liquid. Since the composition of the medium changes during a fed batch experiment, it will have different abilities to dissolve oxygen and O^* often decreases significantly from 100% during an experiment. X is the cell concentration and q_o is the specific oxygen uptake rate. H is Henry's constant, which for water is about 14000 [%l/g] (Åkesson, 1999).

The first term of equation 3.18 is called *the oxygen transfer rate*, and the second part is a measurement of the yeast oxygen consumption.

The dissolved oxygen probe has slow dynamics, which must be taken in consideration. For small deviations from a steady state value, it can be approximated with a first-order system.

$$T_p \frac{dO_p}{dt} + O_p(t) = O(t - t)$$
$$\Rightarrow O_p(s) = \frac{e^{-st}}{1 + sT_p} \cdot O(s)$$

Eq 3.3

O_p is the measured dissolved oxygen tension; t is a time delay in the oxygen sensor and T_p is the approximated time constant. The differential equation above is transformed using the single-sided Laplace transform, which is why the s is introduced in eq. 3.19.

The mass-balance equation can be linearized around an equilibrium point.

$$\Delta O = O - O_0$$
$$\Delta N = N - N_0$$
$$\frac{\partial f}{\partial O} = -K_L a(N)$$
$$\frac{\partial f}{\partial N} = \frac{\partial K_L a}{\partial N}\left(O^* - O\right)$$
$$\Rightarrow$$
$$\frac{d\Delta O}{dt} = -K_L a \cdot \Delta O + \frac{\partial K_L a}{\partial N}\left(O^* - O\right) \cdot \Delta N$$
$$\Rightarrow$$
$$G_{on}(s) = \frac{\frac{\partial K_L a}{\partial N}\left(O^* - O\right)}{s + K_L a} \cdot \frac{e^{-st}}{1 + sT_p}$$

Eq 3.4

3.3.1 Time constant of oxygen sensor

The parameters for the oxygen sensor, t and T_p are not straightforward to determine, since the oxygen sensor is always placed in a liquid and thus the liquid dynamics is always present in an identification of the system. The time delay, t, can usually be neglected. A momentary change of gas from air to nitrogen gas in a medium with no cells will give a step response for the water- and oxygen sensor dynamics together. For a higher stirrer speed, K_La will be higher and the water dynamics will be faster. A step response at maximum stirrer speed will thus give a response where the effect from the water dynamics is minimized.
The time constant measured from the step response is always a little greater than the oxygen sensor time constant. At 1200 rpm the time constant of the oxygen transfer can be measured (from K_La charts, see Figure 3.1) to 5.7s. Since the total

time constant is about 40s, (see Figure 3.2) the oxygen sensor time constant is assumed to be within 35–40s. At 550 rpm, the time constant of the oxygen transfer is measured to 21s and the step response when switching gas will be slower. The total time constant is about 68s.

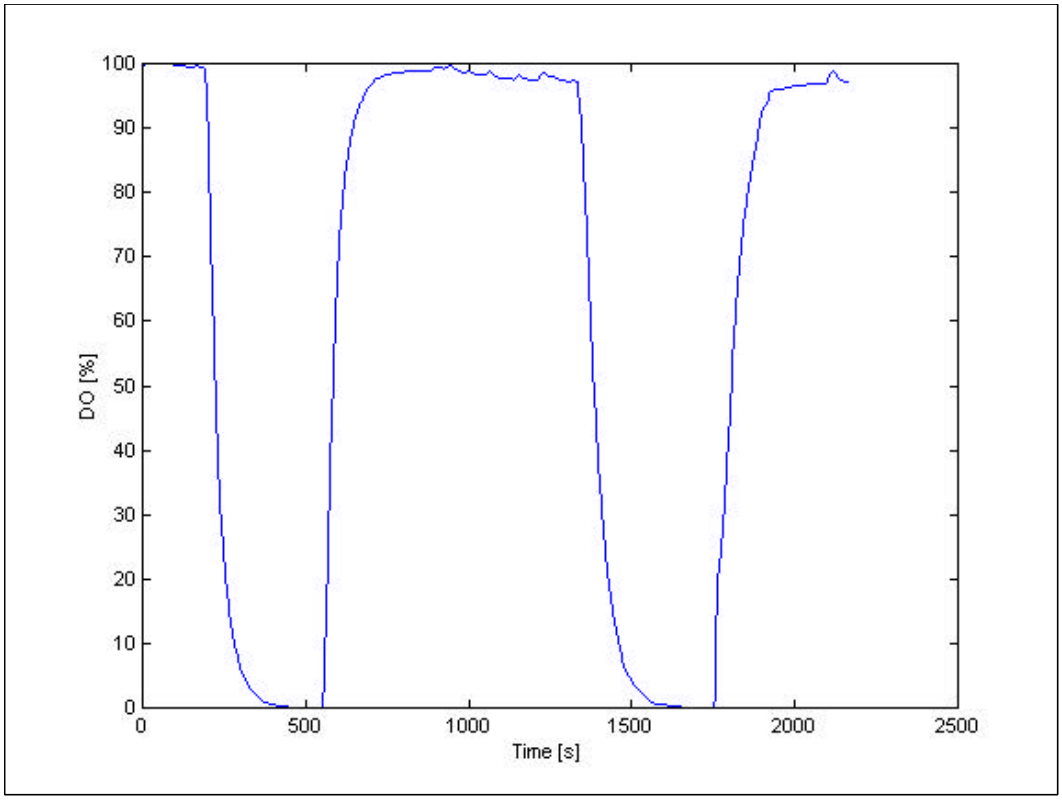

Figure 3.1

Step response for changes between air to nitrogen and back for stirrer speeds 1200 and 550 rpm. At around 1000s the stirrer speed is lowered from 1200 rpm to 550 rpm.

Table 3-1 Parameters used in the model.

Symbol	Value/unit	Description
K_o	0.0001 g/l	Saturation constant for oxygen uptake[1]
K_s	0.1 g/l	Saturation constant for substrate uptake[1]
K_e	0.1 g/l	Saturation constant for ethanol uptake[1]
$q_e^{c,max}$	0.50 g/(gh)	Maximum specific ethanol consumption rate[2]
q_s^{max}	3.5 g/(gh)	Maximum specific glucose uptake rate[1]
q_o^{max}	0.26 g/(gh)	Maximum specific oxygen uptake rate[1]
q_{mc}	0.013 g/(gh)	Maintenance coefficient[3]
$Y_{e/s}$	0.48 g/g	Ethanol/glucose yield[4]
$Y_{o/e}$	1.12 g/g	Oxygen/ethanol yield[4]
$Y_{o/s}$	0.41 g/g	Oxygen/glucose yield for growth[4]
$Y_{o/s}^m$	1.07 g/g	Oxygen/glucose yield for maintenance[4]
$Y_{x/e}$	0.72 g/g	Biomass/ethanol yield[4]
$Y_{x/s}^{ox}$	0.49 g/g	Oxidative biomass/glucose yield[1]
$Y_{x/s}^{fe}$	0.05 g/g	Fermentative biomass/glucose yield[1]
T_p	40 s	Oxygen sensor time constant
O^*	100 %	Dissolved oxygen in equilibrium with air
N	rpm	Stirrer speed
N_0	rpm	Constant ($K_La(N_0)=0$)
α	$(h*rpm)^{-1}$	Constant to describe K_La changes
V_0	1.5 l	Initial reactor volume
X_0	4 g/l	Initial biomass concentration (at start of operation)
E_0	0 g/l	Initial ethanol concentration
S_0	0 g/l	Initial glucose concentration
O_0	95 %	Initial dissolved oxygen concentration
F	0.014 l/h	Feed rate

[1] Values taken from (Sonnleitner, Käppeli et.al. 1986)
[2] Value taken from (S.-O. Enfors Hedenberg, J., Olsson, K., 1990)
[3] Value taken from (Stouthamer, A.H and van Versveld H.W, 1987)
[4] Values calculated from balanced stoichiometric expressions in (Axelsson, 1989)

4 Dissolved oxygen control

To keep the dissolved oxygen concentration in the bioreactor at a level high enough to avoid limitations in the oxygen uptake rate, it must somehow be controlled.
The reactor has two impellers attached to a common axis, which is rotated by a motor. Applying a specified reference voltage to the stirrer control unit can control the stirrer speed to a suitable level. The lower range has to be chosen high enough to maintain a good mixing of the fluid and the highest speed is limited by the maximum speed of the motor.
Beneath the stirrer, a sparger is placed. The sparger is a device that releases the inlet air into the reactor fluid. When the impellers are rotating they give rise to a highly turbulent flow, which will break down the air bubbles into many smaller bubbles, and thus increase the total surface area of the bubbles. For more information about bioprocess technology, see e.g. (Enfors and Häggström, 1998).

The oxygen diffuses through the surface layer of the bubbles and therefore a higher stirrer speed would correspond to a higher volumetric oxygen transfer coefficient, K_La. Figure 3.1 shows how K_La depends on the stirrer speed at two different volumes. At a lower volume, the dependency on the stirrer speed is not as linear due to saturation effects.

4.1 Gain Scheduling

A PI controller with parameters tuned for a low stirrer speed will not perform as well as it could for higher speeds, since K_La will then be higher and it would be possible to increase the gain. It is possible to get a faster controller for higher stirrer speeds but this can be unstable at lower speeds (see Eq. 3.20).
This indicates that it would be preferable to use different controller tunings for different K_La values. Since K_La can approximately be considered to be directly dependent of the stirrer speed (for higher volumes it is a linear dependency), this can be used as a scheduling parameter, i.e. the stirrer speed is used to determine when to change the PI controller. This technique for changing parameters is called gain scheduling even though the integral time changes too. For more information on gain scheduling, see (Åström and Wittenmark, 1995).

The PID controller is implemented by National Instruments in their PID control toolbox for LabVIEW. This toolbox is very useful for PID control applications, and has additional features for gain scheduling and auto tuning. The PID controller uses an integral sum correction algorithm that facilitates anti-windup and bumpless automatic to manual and manual to automatic transfers.

The auto tuning of PI parameters is performed using a setpoint relay experiment of the closed loop system (see figure 4.1). Because of the nonlinear characteristics of the relay, a limiting cycle is generated for most systems. The theoretical reason

for this limiting cycle is shown in (Åström and Wittenmark, 1995). From this cycle the parameters K_u and T_u can be evaluated, where K_u is the ultimate gain and T_u is the ultimate period. The PI parameters are calculated according to a modified (see table 4.1) Ziegler-Nichols heuristic method. Different methods can be chosen, with different characteristics for control. A controller chosen according to table 4.1 is better damped, but slower than a controller chosen according to the original Ziegler-Nichols method.

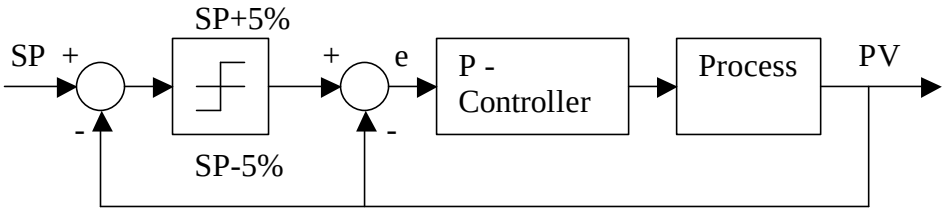

Figure 4.1 The scheme for autotuning experiments. Note that the closed loop process is subjected to the relay experiments.

Table 4-1 Tuning rules used for calculating PI parameters.

Controller	K	T_i
P	$0.2K_u$	
PI	$0.18K_u$	$0.8T_u$

The stirrer controller was tuned in this manner. To vary the different tuning stirrer speeds, the glucose feed rate was varied. The parameters were tuned for a low reactor volume. In table 4.2, the resulting parameters are shown for a volume of 1.4 liters.
Since the slope of the K_La curve decreases for higher stirrer speeds and the absolute K_La value increases, the controller should have a higher gain for those speeds. This can easily be seen in the Bode diagrams (Figure 4.2 and figure 4.3). The diagrams describe the linearized model in Eq. 3.20 with the two expressions for K_La from figure 3.1. For a low reactor volume, the magnitude of the transfer function for 1100 rpm is significantly smaller than that of 800 rpm. This means that we can have a higher gain for high stirrer speeds to keep the magnitude of the closed loop system approximately constant in the low frequency range.
In the case where the reactor is full, the difference in magnitude between different stirrer speeds can still be noticed, but it is much smaller.

Table 4-2 Tuned PI parameters

Stirrer speed used to tune parameters	K	T_i (minutes)	Comment
645	3.89	2.05	
750	4.41	1.72	
775	6.42	1.61	slightly higher volume
820	7.45	1.54	slightly higher volume
930	10.6	1.59	
1025	13.4	1.52	

Comparisons of the tuned PI parameters have been made for one stirrer speed when the reactor was full. For a stirrer speed of 1060 rpm the tuned parameters were K=9.93 and Ti=1.62 min.
The difference can be explained by the fact that the slope of K_La is larger for high stirrer speeds when the reactor is full.

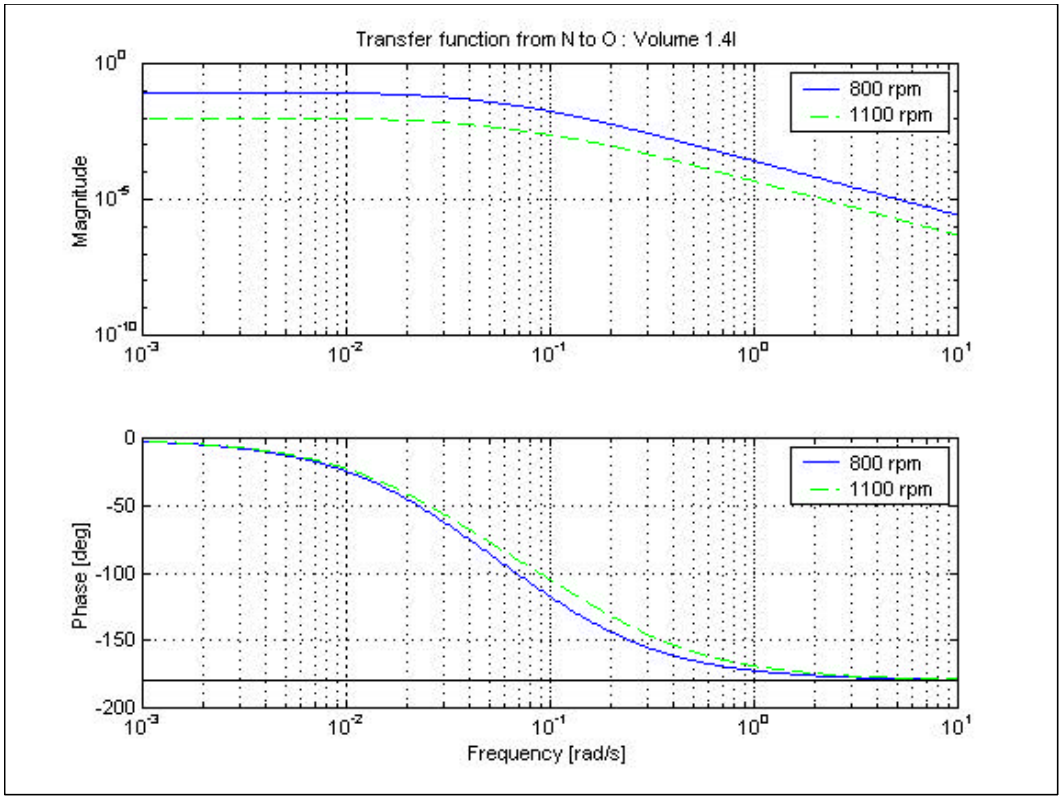

Figure 4.2 Bode diagram for a medium volume of 1.4l.

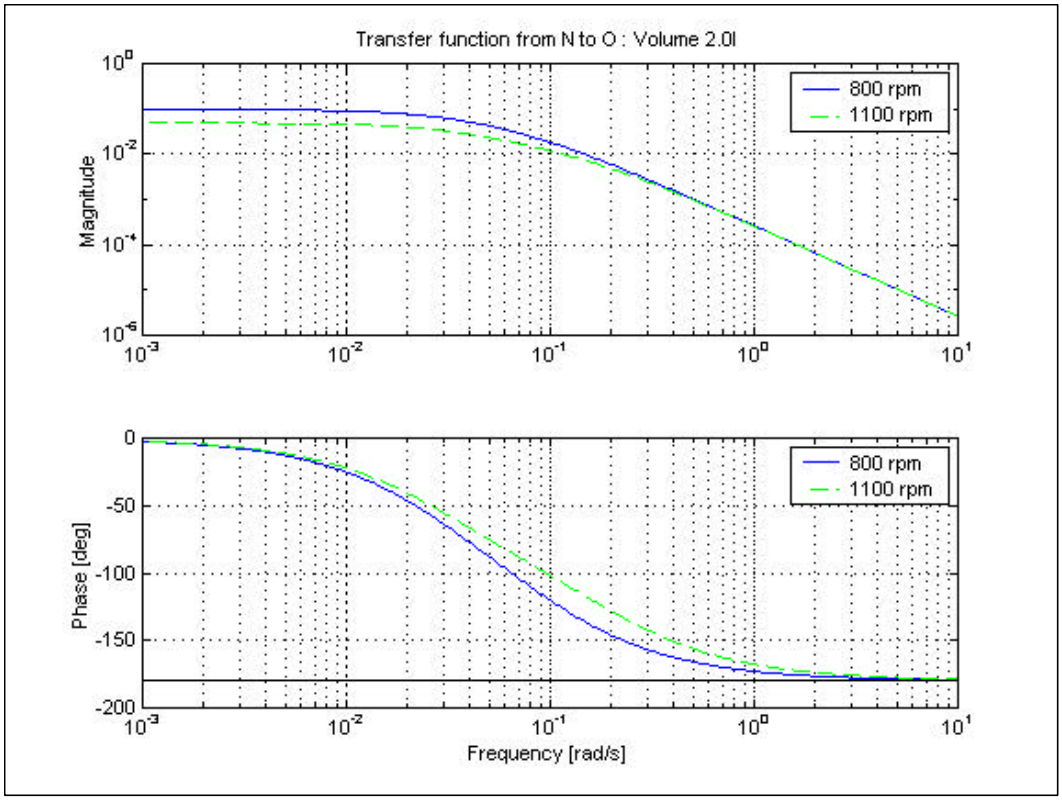

Figure 4.3 Bode diagram for a medium volume of 2.0l.

5 Fed-batch probing control theory

To maximize the growth of biomass, it's important to avoid overflow metabolism, and thus ethanol production, but still keep the feed rate as high as possible throughout the fedbatch. The theoretical feed rate needed to maintain a constant specific growth rate is exponentially increasing with time, assuming a sufficient oxygen transfer can be maintained.

$$F(t) = \frac{q_s \cdot (VX)_0 \cdot e^{mt}}{S_{in} - S(t)}$$

According to the model, ethanol production does not occur while $q_s < q_s^{crit}$, assuming that the dissolved oxygen tension is large enough, and thus it seems like a good idea to keep the glucose uptake rate as close as possible to the critical uptake rate. To avoid oxygen depletion in the medium, the cells must never use more oxygen than what is provided at the maximum oxygen transfer rate in the reactor. Controlling the speed by which the glucose is added to the reactor can achieve this.

5.1 Avoiding overflow production of ethanol

If the specific glucose uptake rate, q_s, is larger than the critical glucose uptake rate, q_s^{crit}, ethanol will be produced and the specific oxygen uptake rate, q_o, will be approximately constant, even if q_s increases further (see figure 5.1).

Relations between specific oxygen uptake rate, specific ethanol production and specific glucose uptake rate are shown in the figure. Dash-dotted lines show what happens when ethanol has been accumulated in the reactor. The specific oxygen uptake rate will then be saturated for a lower specific glucose uptake rate, since additional oxygen is needed for the ethanol consumption. Ethanol consumption can only occur when the specific glucose uptake rate is below the critical glucose uptake rate, according to the model.

To determine if $q_s < q_s^{crit}$ a pulse in the feed rate is given. This pulse should give an increasing oxygen consumption rate, which can be observed in the dissolved oxygen tension. If there is any free respiratory capacity, the feed rate will be increased in order to use it all.

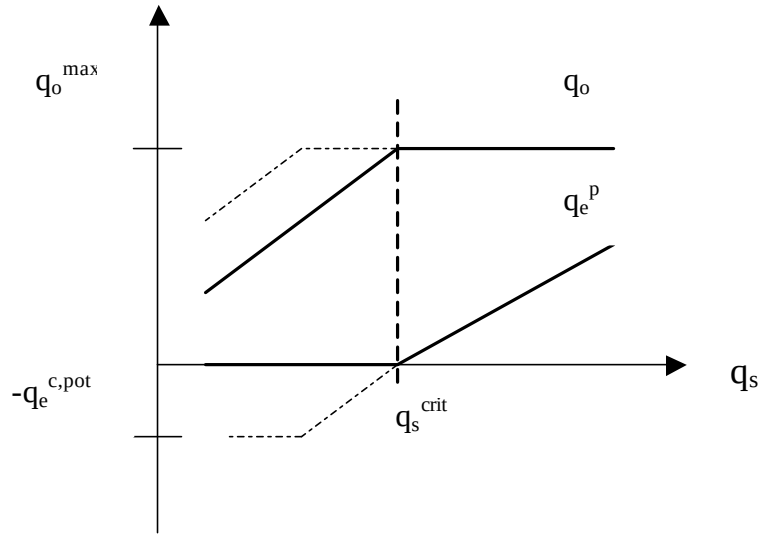

Figure 5.1 Relation between q_o and q_s. Above a certain q_s value, q_o will be saturated. When any ethanol has accumulated in the media, q_o will be saturated for a lower q_s. The dash-dotted lines show what the relations are with some ethanol present in the media.

The probing controller used in this thesis is based on the simplified probing controller (Åkesson and Hagander, 2000) that uses up-pulses in the glucose feed. The analysis of the system is taken from (Åkesson and Hagander, 2000) and (Åkesson, 1999). During a pulse, a change in the feed rate increases the glucose concentration in the reactor. The specific glucose uptake rate is related to the glucose concentration, and q_s will increase. When the specific oxygen uptake rate is not saturated, a response can be observed in the dissolved oxygen tension. After the pulse, the controller tries to adjust the feed rate to keep the glucose concentration such that q_s is kept below, but still close to the point where the specific oxygen uptake rate is saturated.
If some ethanol has accumulated, the point in q_s where q_o will be saturated is lower and no response in the dissolved oxygen tension may be observed. The feed rate will then be lowered and thus the ethanol consumption rate should increase. This means that the ethanol concentration in the reactor will be kept low during the cultivation.

The oxidative biomass yield of glucose is much larger than the fermentative biomass yield of glucose and therefore it's always preferable to have oxidative growth if the goal is to maximize the biomass yield.
The controller gives the maximum growth rate possible without overflow metabolism, since q_s is always kept very close to q_s^{crit}.

5.2 The control algorithm

The probing controller algorithm calculates the maximum feed rate possible to ensure that the fermentative growth is minimized, preferably avoided.
Each cycle consists of a pulse, where the feed rate is increased momentarily and a following control period where the feed rate is changed from what it was during the last control period. The changes depend on the oxygen response from the pulse. During the control period, the dissolved oxygen tension is maintained at its setpoint using a PID controller, by manipulating the stirrer speed.

The control strategy of the stirrer is to control the DO during control periods and keep the speed constant during each pulse and thus keep the oxygen transfer constant during that time. If the oxygen transfer is kept constant during the pulse, the dissolved oxygen tension may decrease and if it decreases below a specified level, it is determined that $q_s < q_s^{crit}$, and the feed rate will be increased after the pulse.
If no response in the dissolved oxygen can be observed, q_o has been saturated. As shown before, q_s is then larger than q_s^{crit}, or ethanol has been accumulated and additional oxygen is needed for consumption. To free some respiratory capacity, the feed rate can be lowered with a fixed amount. The ethanol produced during the pulse will, hopefully, get consumed during the following control period. Experiments have been done to verify this.

5.2.1 The safety net

When the maximum stirrer speed has been reached, the DOT might approach zero. To avoid a situation where the oxygen consumption is larger than what can be transported into the reactor, some adjustments in the feed rate can be made. This typically happens after a couple of hours in the fed batch phase. The fed batch phase can thus be divided into two different parts, a first part, where the pulses determine the feed rate and a second part where the feed rate is limited by the oxygen transfer.
When the stirrer speed approaches its maximum value, the situation gets critical and the feed rate can be lowered to decrease the oxygen consumption. This can be done by including two different limit levels. When the stirrer speed is above the lower (90% of the maximum stirrer speed) limit during the pulse, no increments of the feed rate are allowed, no matter what the pulse responses were. If the stirrer speed gets above the second limit (95% of the stirrer speed) any time between pulses, the feed rate is lowered continuously until the stirrer speed has gone below this limit. The decrements are done every time the controller is executed from the main program. $T_{control}$ is the duration of the control period and h is the sampling period of the probing controller.

$$F_{control}(k) = F_{control}(k-1) - \frac{g_l \cdot h}{T_{control}} \cdot F_{control}(k-1)$$

Eq 5.1

If the stirrer speed is above the upper limit during the whole control period, the feed rate will be decreased with an amount that is approximately one pulse height, for a ?$_l$ that equals ?$_p$. ?$_l$ has been kept smaller than- or equal to ?$_p$ in the experiments.

The stirrer speed must not be less than some minimum stirrer speed, to maintain a good mixing of the medium. An equal safety net with two lower limits has been implemented as well. The adjustments are the same, with the difference that the feed rate is increased until the stirrer speed has reached appropriate values.

5.3 Pulses

The pulse height has to be large enough to give a detectable response in the dissolved oxygen tension but small enough to ensure that no fermentative growth will occur due to lack of oxygen. To determine if the pulse response is large enough, it must exceed a reaction level, O_{reac}.
When subjected to a step with amplitude F_{pulse} in the feed rate, assuming that there is no ethanol in the medium, the steady state response in DO is given by:

$$\left|O_{step}\right| = \left|K\right| \cdot F_{pulse} = \frac{O^* - O_{sp}}{1+a} \cdot \frac{F_{pulse}}{F_{control}}$$

Eq 5.1

according to the linearization, Eq 3.16.

Since the pulses have a limited length, the response in DO may not reach the steady state response described above. The pulses do, however, have a length that is longer than or equal to the overall time constant of the system. This means that the amplitude of the pulse response will always exceed 0.63*A, where A is the steady state response of the system.

$$\left|O_{pulse}\right| = \left|K\right| \cdot F_{pulse} = b \cdot \frac{O^* - O_{sp}}{1+a} \cdot \frac{F_{pulse}}{F_{control}}$$

$$0.63 \le b < 1$$

Eq 5.2

We can use a proportional increment of $F_{control}$ to get F_{pulse}.

$$F_{pulse} = g_p \cdot F_{control}$$

Eq 5.3

The maximum response in DOT occurs when a=0 and ß=1.

$$\left|O_{pulse}\right| \le g_p\left(O^* - O_{sp}\right)$$

Eq 5.4

If $?_p$ is chosen to large, the dissolved oxygen level will approach zero during the pulse and then fermentative growth will occur, with a large ethanol production as a by-product. In all fed-batch experiments, $?_p = 0.1714$ has been used and no extremely low DOT values have been observed during any of the pulses.

5.4 Stability conditions

Since the pulse duration, T_{pulse}, is chosen to be the overall time constant of the system at low cell densities, the glucose dynamics is quickly approaching a steady state value during the pulses.
From equation 3.14, the glucose uptake rate can be approximated by

$$q_s^{pulse} = K_s \cdot F_{pulse}$$

Eq 5.1

A response in DOT depends on whether q_s is below or above q_s^{crit} before the pulse is issued. If q_s is far below q_s^{crit} such that the specific glucose uptake rate after the pulse is below q_s^{crit}, the response is

$$\left|O_{pulse}\right| = b \cdot \left|K_o\right| \cdot q_s^{pulse}$$

Eq 5.2

If part of the pulse is above q_s^{crit}, only the part that is below can be observed as a response in DOT.

$$\left|O_{pulse}\right| = b \cdot \left|K_o\right| \cdot \left(q_s^{crit} - q_s\right)$$

Eq 5.3

If q_s is above q_s^{crit} even before the pulse is given, no response in DOT can be observed.

Assuming a response has been observed, a lower bound for the distance between q_s and q_s^{crit} can be obtained.

$$q_s^{crit} - q_s \ge \frac{\left|O_{pulse}\right|}{\left|K_o\right|}$$

Eq 5.4

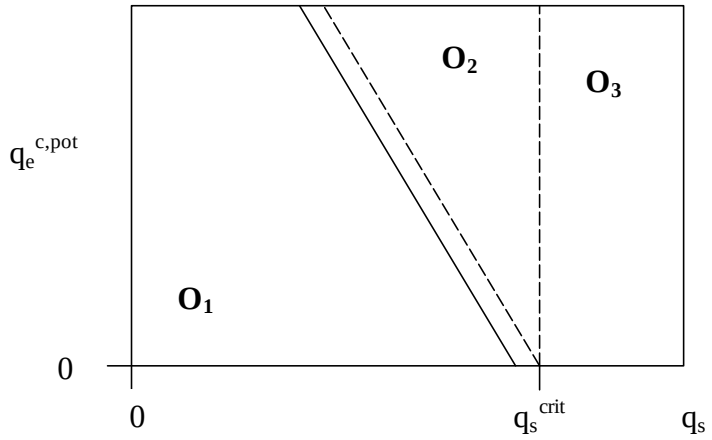

Figure 5.2 Plane for stability analysis. The area between the dashed lines is where the oxygen uptake is saturated.

If any ethanol has accumulated, it can be consumed if $q_s < q_s^{crit}$ (figure 5.1). The specific oxygen uptake rate will then be saturated for a lower value of q_s. Since q_o may be saturated for $q_s < q_s^{crit}$, it affects the DOT responses. A plane, which describes this phenomenon, will be used in the analysis. The axes are q_s and $q_e^{c,pot}$ (fig. 5.2).

In the plane, three different regions are chosen.

- Region O_1, where q_o is never saturated and thus gives responses to pulses every time.
- Region O_2, where no sufficiently large response to pulses can be observed. The solid line to the left, and the dashed line at q_s^{crit} delimit the region. In the region between the two dashed lines, any accumulated ethanol will be consumed. q_o will be saturated everywhere. In the area between the solid and dashed lines, part of the pulse will give a small response but not a sufficiently large one. The amplitude of the response will be less than the reaction level.
- Region O_3, where ethanol production always will occur, due to the respiratory limitation when $q_s > q_s^{crit}$. Since this is a region with respiratory limitations, no pulse responses can be observed here either.

5.5 Control of the glucose feed in the reactor

The closed loop system where the probing controller is connected to the process is examined. In the plane, parameters before each pulse is plotted and connected. The trajectories will be considered for a stability analysis.
The glucose dynamics has a time constant, which is much faster than the duration of the control period. This means that the effect from the pulses can be neglected after the control period. From the system linearization, equation 3.14, ? q_s can be written as

$$\Delta q_s(k) = K_s \cdot \Delta F_{control}(k)$$
$$q_s(k+1) = q_s(k) + \Delta q_s(k) = q_s(k) + K_s \cdot \Delta F_{control}(k) - e$$

Eq 5.1

e is a factor, which denotes the influence from increasing cell mass to q_s. In the following discussions, e will be neglected.

Since there will be responses in DOT from the pulses in region O_1, ? F will be positive in this region and thus q_s will increase.
In regions O_2 and O_3, ? F will be negative since no responses of the pulses will be detected. This means that from regions O_2 and O_3, q_s will decrease.
When $q_s < q_s^{crit}$, the accumulated ethanol will be consumed. $q_e^{c,pot}$ will therefore decrease, or remain zero, in regions O_1 and O_2.

To avoid oscillation between increments and decrements of the feed rate, it is important to prevent jumps from region O_1 to region O_3. If ? q_s is too large, oscillations will occur. To avoid oscillations, it is important to keep ? q_s smaller than the shortest distance from region O_1 to region O_3.

In Eq 5.9 the lowest bound for distance q_s^{crit} - q_s for a given response is shown. Since this is the shortest distance between the O_1 and O_3 regions, the change in q_s must be less than this value.

$$\Delta q_s \le \frac{|O_{pulse}(k)|}{|K_o|}$$

Eq 5.2

Replacing ? q_s according to Eq. 5.10, an expression for ? F can be derived.

$$\Delta F(k) \le \frac{|O_{pulse}(k)|}{K_s \cdot |K_o|} \le |O_{pulse}(k)| \cdot \frac{1+a}{O^* - O_{sp}} \cdot F_{control}(k)$$

Eq 5.3

Considering the worst case, a=0, a maximum allowed increment has been derived.

$$\Delta F(k) \leq \frac{\left|O_{pulse}\right|}{O^* - O_{sp}} \cdot F_{control}(k)$$

Eq 5.4

To have a margin to instability, a gain, ?, is introduced where stability is guaranteed for ? = 1. It is possible however, to have a faster control with higher ? value.

$$\Delta F(k) = \frac{k * \left|O_{pulse}(k)\right|}{O^* - O_{sp}} \cdot F_{control}(k)$$

Eq 5.5

For the proportional controller (no integral part) implemented in LabVIEW, no increments above the pulse height are allowed. This is done to avoid that the calculated increment sometimes gets too large and causes oscillations.

If no response large enough can be detected, the feed rate is decreased with sixty percent of the pulse height. This value can be altered but in most fed-batch experiments included in this thesis, sixty percent has been used. The reason for this is to let it be large enough to enable the cells to consume accumulated ethanol if there is any.

$$\Delta F(k) = -0.6 \cdot g_{p} \cdot F_{control}(k)$$

Eq 5.6

A comparison between two simulated controllers with different gains shows that one system is oscillating between different regions and one system is a stable system. See figures 5.3 and 5.4. The dashed lines indicate the region where the specific oxygen uptake rate has been saturated. Both simulations use an initial cell mass concentration of 4 g/l.

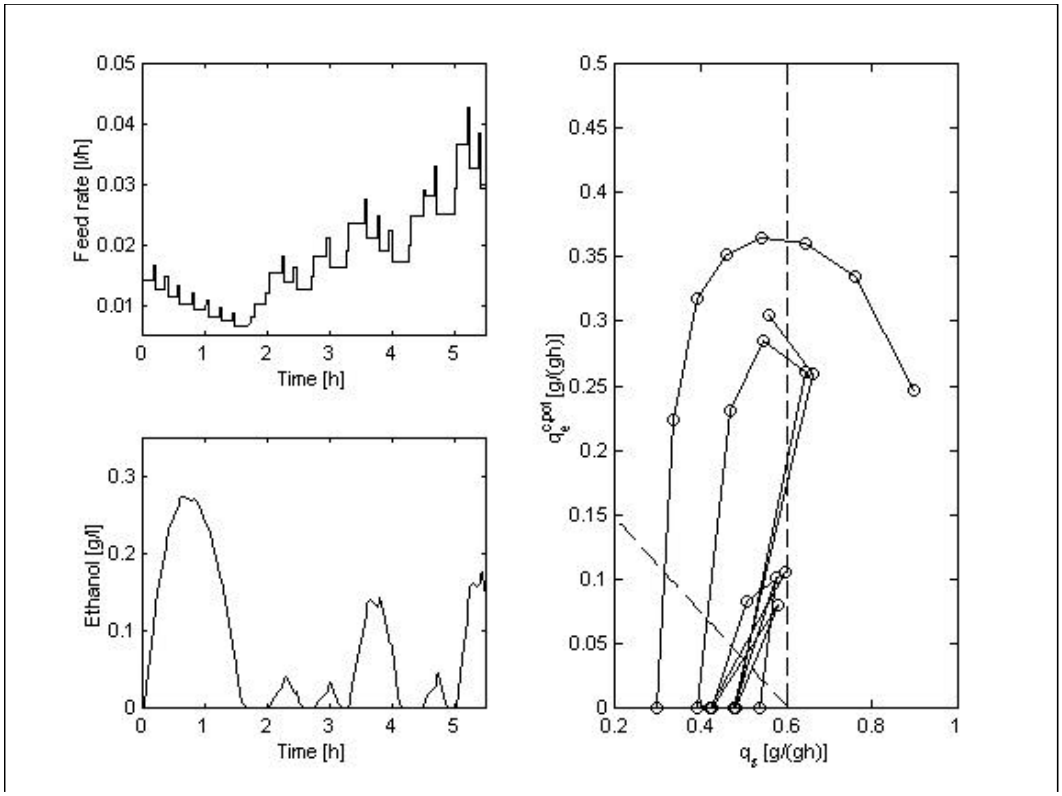

Figure 5.1 Kappa = 4. The controller is oscillating between increments and decrements.

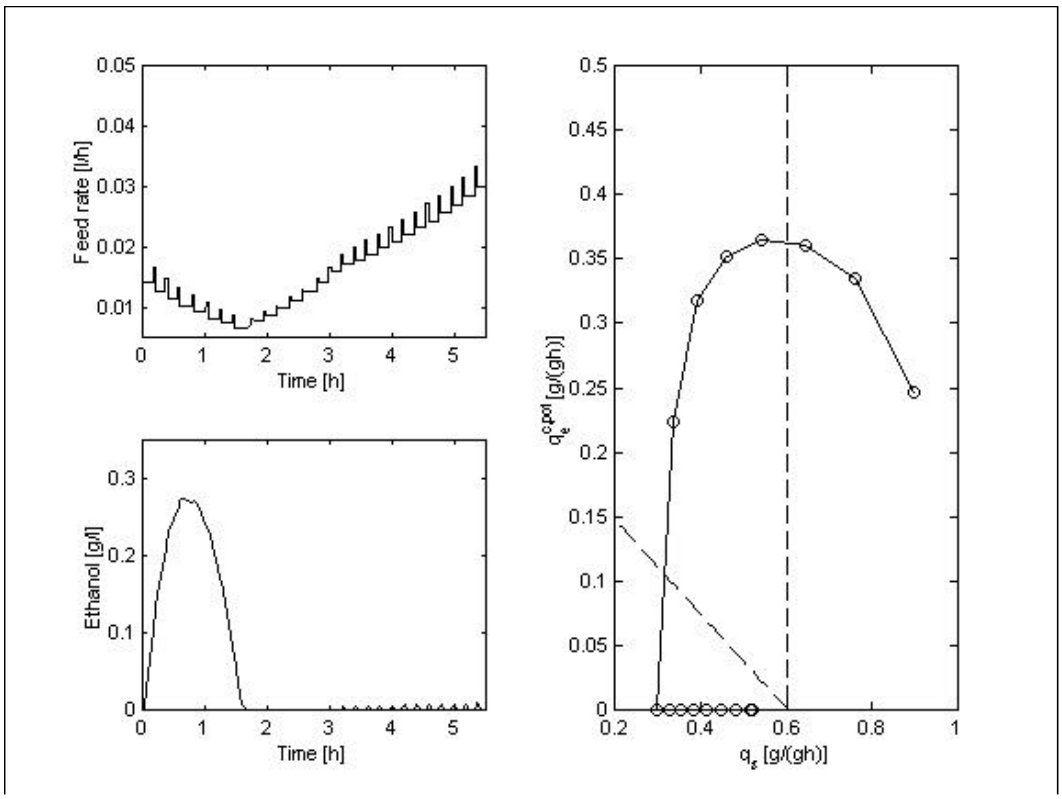

Figure 5.2 Kappa = 1. The controller is stable and approaching a stationary point.

5.6 Integral action

None of our fed-batch experiments have been controlled with a controller using an integral part but the theory will still be included here.

Even if the proportional gain, ?, is less than one, the system can show oscillatory behavior. This can happen if q_s^{crit} decreases during the cultivation, see chapter 8.
To have a shorter way of writing O_{pulse}, it will now be called y. From Eq. 5.8, where part of the pulse will cause a glucose uptake that is above the critical uptake rate, it can be written

$$y(k) = b \cdot |K_o| \cdot \left(q_s^{crit} - q_s\right)$$

Eq 5.1

Using Eq. 5.10 this gives

$$y(k+1) = y(k) - b \cdot \underbrace{|K_o| \cdot K_g}_{|K|} \cdot \Delta F_{control} + b \cdot |K_o| \cdot e$$

Eq 5.2

Using Eq. 5.17 and 5.14 we get

$$y(k+1) = \left(1 - \frac{bk}{1+a}\right) y(k) + b \cdot |K_o| \cdot e$$

Eq 5.3

This difference equation converges to a stationary point if its characteristic polynomial has it's pole within the unit circle, that is if 0 < ? < 2(1+a)/ß. The convergence point will be

$$y^* = \frac{(1+a) \cdot |K_o| \cdot e}{k}$$

Eq 5.4

If this stationary point becomes too low, i.e. below O_{reac}, The controller will oscillate between increments and decrements. Introducing a setpoint in the control law will change the stationary point, and

$$\Delta F(k) = k \cdot \frac{y(k) - y_r}{O^* - O_{sp}} \cdot F_{control}$$

Eq 5.5

gives the new stationary point,

$$y^* = \frac{(1+a)\cdot|K_o|\cdot e}{k} + y_r$$

Eq 5.6

For $y_r > O_{reac}$, the stationary response will exceed the reaction limit but if it is chosen too large, q_s will decrease below the proportional band. This must be avoided since the control theory has been developed for the proportional band.

An augmented method is to use an integral part in the probing controller.

$$\Delta F(k) = \left(k\left(y(k) - y_r\right) + k_i \sum_{j=0}^{k} \left(y(j) - y_r\right) \right) \cdot \frac{F_{control}(k)}{O^* - O_{sp}}$$

Eq 5.7

Inserting this control law in Eq. 5.17 an expression for y(k) will be obtained. If it is assumed that the sampling periods have equal length then the expression can be transformed into the z-plane.

$$Y(z) = \frac{\frac{b}{a+1}\left(\left((k+k_i)Y_r(z) + (1+a)|K_o|e\right)z - \left(kY_r(z) + (a+1)|K_o|e\right)\right)}{z^2 + \left(\frac{b(k+k_i)}{a+1}\right)z + \left(1 - \frac{bk}{a+1}\right)}$$

Eq 5.8

A condition for stability is that both of the poles are within the unit circle of the z-plane. Using Jury's Criterion can show this (Åström and Wittenmark, 1997). The conditions on the parameters ? and $?_i$ for stability are

$$k_i > 0$$
$$k > 0$$
$$k_i + 2k < \frac{4(a+1)}{b}$$

Eq 5.9

Multiplying Y(z) with $(1-z^{-1})$ and letting z? 1, according to the final value theorem, we can se that the convergent point of y(k) will be the same as the set point, if the setpoint is chosen as a step with amplitude y_r.

$$y^* = y_r$$

Eq 5.10

If the ? $F_{control}$ calculated by the control law in Eq. 5.22 becomes too large, the integral part is changed to fulfill the stability condition in Eq. 5.13.

6 Material and methods

6.1 Experimental setup

Experiments were performed in the laboratories at Chemical Engineering II, Lund Institute of Technology. The equipment used is specified in table 6.1.

Table 6.1 The equipment used

micro-DCU-300	bench-top digital measurement and control system from B. Braun Biotech International.
Pump-300	Pump system for bench scale bioreactor from B. Braun Biotech International.
MCU-200 BIOSTAT® A.	Stirrer Control Module for the fermentor system and Stirrer Drive Motor for the BIOSTAT® A from B. Braun International.
InPro® 6000 T-96	12mm Polarographic O_2 Sensor from Mettler Toledo.
He?ios Alpha	UV-Visible Spectrometer from Unicam Instruments.
Industrial Emissions Monitor Type 311	Gas analyzer from Brüel & Kjær.
PCI-6024E	Low cost multifunction I/O board from National Instruments.
LabVIEW 5.0	Graphical Programming for Instrumentation form National Instruments.
Alitea XV	Peristaltic pump from Watson-Marlow Alitea AB.
F-201C-FA-33-V	Mass Flow Controller from Bronkhorst Hi-tech.
3 liter fermentor	B. Braun Biotech International

The experiments were carried out using the simplified probing controller (Åkesson et. al., 2000), described in the previous chapter, implemented in LabVIEW. The micro-DCU-300 control system was used to control reactor temperature to 30ºC and the pH level to 5.0 by addition of 2 M NaOH. It was also used to collect values of dissolved oxygen tension and pH-level for monitor purposes in LabVIEW. Foaming was avoided by automatic addition of Antifoam 289 when needed. The stirrer speed was controlled by a software PID controller, available in the PID Control Toolkit for G, which is a stand-alone toolkit for LabVIEW, available from National Instruments.
The toolkit contains programs for auto tuning and gain scheduling PID controllers, which are very useful in this control problem. To vary the feed rate, a subprogram calculated an appropriate flow and the signal was sent to the pump which added substrate solution to the reactor.

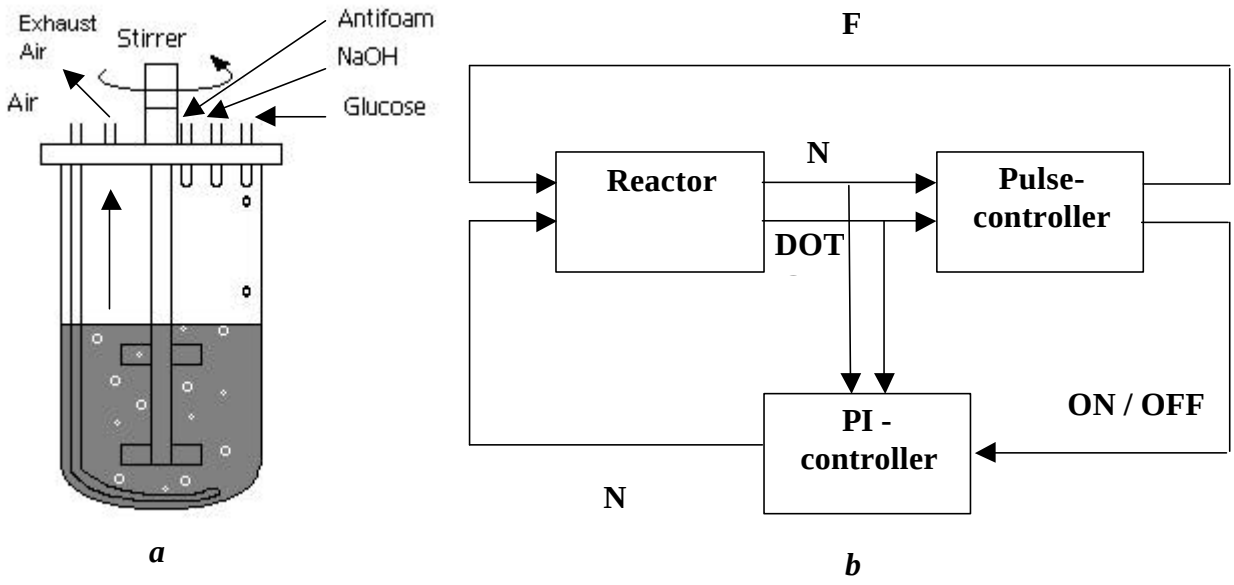

Figure 6.1 a) A schematic picture of the reactor system. b) A block scheme of the closed-loop system.

In some of the experiments (both batch and fed-batch phase), the exhaust gas was analyzed for oxygen, carbon dioxide and organic components, using a gas analyzer.

6.2 Fed-batch cultivations

All cultivations consisted of three phases. At first an inoculum phase, to give an initial increase in cell mass concentration. A batch cultivation in a larger scale followed, in order to give a second boost in cell mass concentration. The last and most important procedure was the fed-batch cultivation, which was done with or without pulse feeding.

6.2.1 Yeast strains and media

Two commercial strains of Bakers's yeast were used, one called "red" and one called "blue". Both strains were obtained from Jästbolaget AB, Rotebro, Sweden. The "red" strain is mainly for use in sweet doughs, while the "blue" one works best in ordinary doughs.

The medium for the fermentor contained a nitrogen source, $(NH_4)_2SO_4$, and other salts in order to satisfy the cells demand for N, S, P, Mg, K, and trace metals. The carbon source used was glucose.

Table 6.2 Salt solution added to the fermentor medium, in order to satisfy the cell demand for N, P, S, K, Mg, and trace metals.

Standard solutions	*Amounts added*
$(NH_4)_2SO_4$ (1.14 M)	225 ml
K_2HPO_4 (1.03 M)	113 ml
$MgSO_4$ (0.77 M)	36 ml
Trace metal solution*	45 ml

* The composition can be seen in Appendix H.

The salt solution (seen in table 6.2) was diluted to 1.2 l with distilled water. pH was adjusted to 5.0 with 2M NaOH. The media was poured into the reactor, which was autoclaved at 120ºC for 20 min. Then 11 ml of vitamin solution (Appendix H) and 6 drops of antifoam 289 was added.

6.2.2 Inoculum preparation

Table 6.3 Salt solution added to the inoculum medium, in order to satisfy the cell demand for N, P, S, K, Mg, and trace metals.

Standard solutions	*Amounts added*
$(NH_4)_2SO_4$ (1.14 M)	5 ml
K_2HPO_4 (1.03 M)	2.5 ml
$MgSO_4$ (0.77 M)	0.8 ml
Trace metal solution	1ml

The salt solution (seen in table 6.3) was diluted to 100 ml and autoclaved for 20 min in 120ºC. Afterwards, 0.1 ml sterile-filtered vitamin solution was added. pH was adjusted to 5.0 with 2M NaOH. Approx. 0.1 g of yeast culture from an agar plate was added. The flask was then incubated at 30ºC for ca. 24 h.

6.2.3 Batch phase cultivations

The experiments started with an initial batch cultivation phase intended to give a biomass conc. of about 12 g/l.

"Blue" yeast: 59 g glucose dissolved in 110 ml distilled water (the glucose solution was autoclaved together with the reactor), was added to the reactor (with media). At last 50 ml of inoculum was added via a septum.

"Red" yeast: Instead of 59 g glucose, 75 g (in batch 13 and B, 59g) were added in the same way as before.

The batch cultivations lasted for at least 35-40 h and were interrupted when the DOT had reached a plateau level above 90 %. The stirrer speed was 700 rpm.

6.2.4 Fed-batch phase with pulse feeding

Seven successful fed-batch experiments with pulse feeding were made, five with the "blue" yeast (8, 9, 10, 11 and 17) and two with the "red" yeast (15 and 16). Mixing a glucose and a salt solution after sterilization made the feed solution for the fed-batch phase.

Table 6.4 The composition of the salt solution added to the feed solution, in order to satisfy the cell demand for N, P, S, K, Mg, and trace metals during the fed-batch cultivation.

Standard solutions	*Amounts added*
$(NH_4)_2SO_4$ (1.14 M)	265 ml
K_2HPO_4 (1.03 M)	133 ml
$MgSO_4$ (0.77 M)	43 ml
Trace metal solution	53 ml

"Blue" yeast: 332 g of glucose were diluted in distilled water to a final volume of 420 ml. After sterilization the salt solution (seen in table 6.4) was added. Final glucose concentration: 400 g/l

"Red" yeast: 332 g of glucose were diluted in distilled water to a final volume of 600 ml. After sterilization the salt solution (seen in table 6.4) was added. Final glucose concentration: 325 g/l

The initial feed rate was set at about 0.013 l/h and the first pulse was given when DOT had been stabilized around 30%.
In order to avoid extensive foaming a foam probe in the reactor was connected to the fermentation unit enabling additions of antifoam 289 by activating the pump connected to the fermentation unit.

6.2.5 Fed batch experiments without pulse feeding

Two fed-batch cultivations, one with “blue” (A) and one with “red” yeast (B), were made without applying pulse feeding. The initial feed rate, F_0, was 0.014 l/h and increased exponentially according to the function $0.014 \cdot e^{0.12t}$ (l/h). The DO was held at 30 % and when N reached 1200 rpm the feed profile was changed to a slightly decreasing linear curve, $F_1 - 2.78 \cdot 10^{-4} \cdot t$ (l/h).
The glucose concentration in the feed solution was 400 g/l. Except for this the procedure was the same as during the pulse feeding experiments.

6.2.6 Analyzes

Samples for cell mass were taken every two hours (not during the batch phase).
Two test tubes were filled with 10 ml of media and centrifuged at 4000 rpm for 3 min. The solution was then discarded and the pellet was washed with 4 ml of water and then centrifuged again. The solution was removed and the pellet was dried at 105° C for 24 h.

Additional samples for glucose and ethanol determination were taken every hour (not during the batch phase). Two Eppendorf tubes were filled with media and immediately centrifuged at 13,000 rpm for 4 min. The solution was transferred to new tubes and was stored in -20° C until time of analysis.
The concentrations were determined using commercial enzymatic kits.
Test kits used were:

Ethanol: 0176290, Boehringer Mannheim

Glucose: 716 251, Boehringer Mannheim

On line gas analyzis with a gas analyzer was also made during some of the fed-batches (9, A and B).

7 Results

7.1 Batch growth

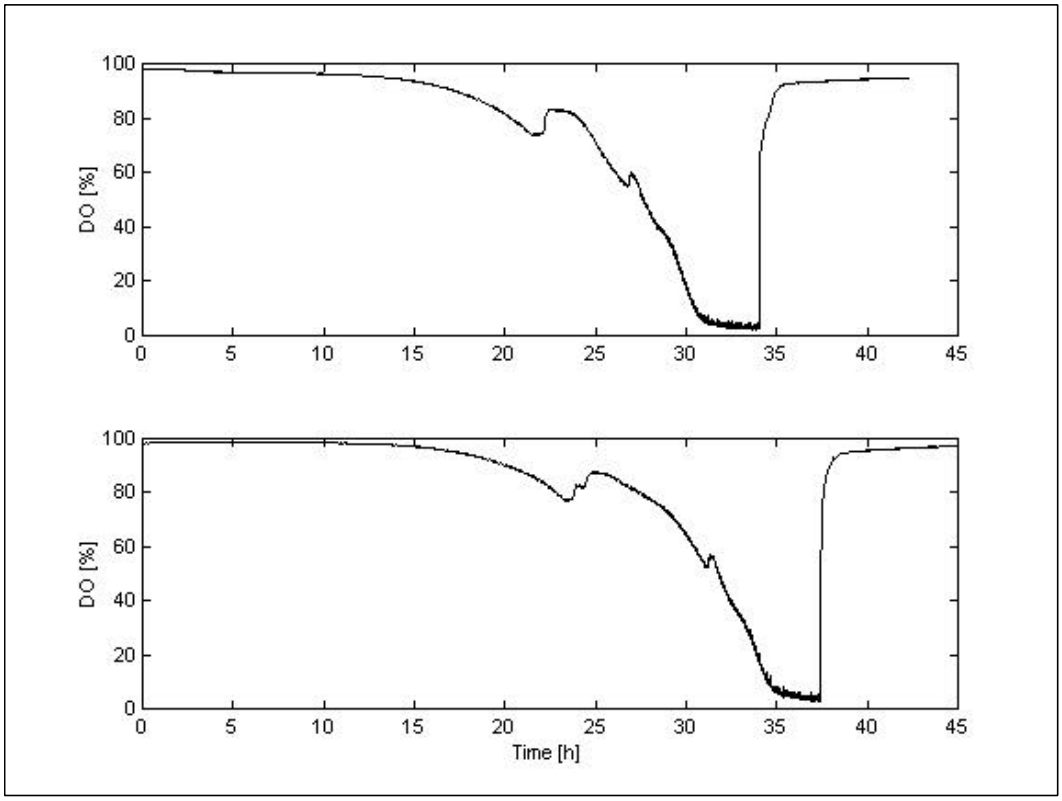

Figure 7.1 The figure shows the DOT during batch-A ("blue" strain, top panel) and batch-B, ("red" strain, lower panel), typical batch cultivations of the strains used. The curves are indicative of a diauxic growth with a glucose consumption phase, which lasted for the first 22-23 h, followed by an ethanol consumption phase. The glucose consumption phase was shorter for the "blue" yeast, 22 h, compared to 24 h for the "red". The ethanol consumption phase was also shorter for the "blue" yeast; ca 11 h compared to 13 h for the "red" yeast.

An initial batch phase cultivation preceded all the fed batches. Its purpose was to increase the cell mass density to about 12 g/l. The stirrer speed was kept constant at 700 rpm.
The overall biomass yields (after complete consumption of both glucose and ethanol) were:

$\mathbf{Y_{x/s}}$ ("blue" yeast) 0.30 ± 0.02 g/g
$\mathbf{Y_{x/s}}$ ("red" yeast) ca 0.28 g/g
(59 glucose were added, no s (standard deviation) could be calculated due to the few values, the yields were obtained during batch 13 and B)
$\mathbf{Y_{x/s}}$ ("red" yeast) 0.26 ± 0.02 g/g
(75 glucose was added, the yields were obtained during batch 14, 15 and 16)

7.2 Fed-batch phases

Only two complete fed-batch experiments will be included in this chapter. The rest can be found in Appendix A.

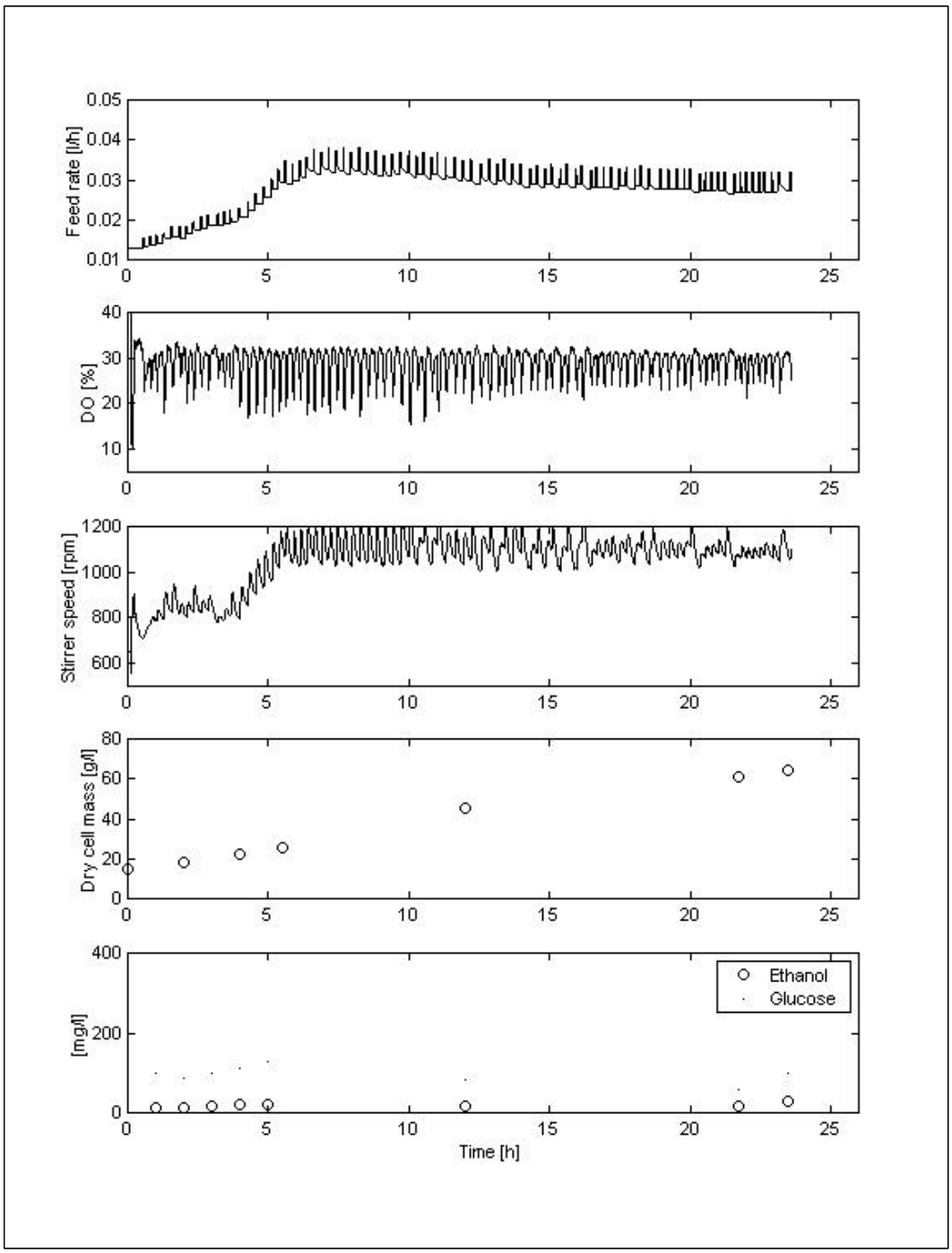

Figure 7.1 Fed-batch 17. Example of a fed-batch phase, where the “blue” strain was used.

Examples of fed-batch cultivations (pulse feeding) with both strains are shown in figure 7.1 and 7.2. There are two main phases in the fed-batch cultivations. First an initial phase in which the feed rate increases exponentially. After about 7 h the oxygen transfer saturates, and due to the safety net in the regulator the feed rate is slowly decreased during the rest of the fed-batch.

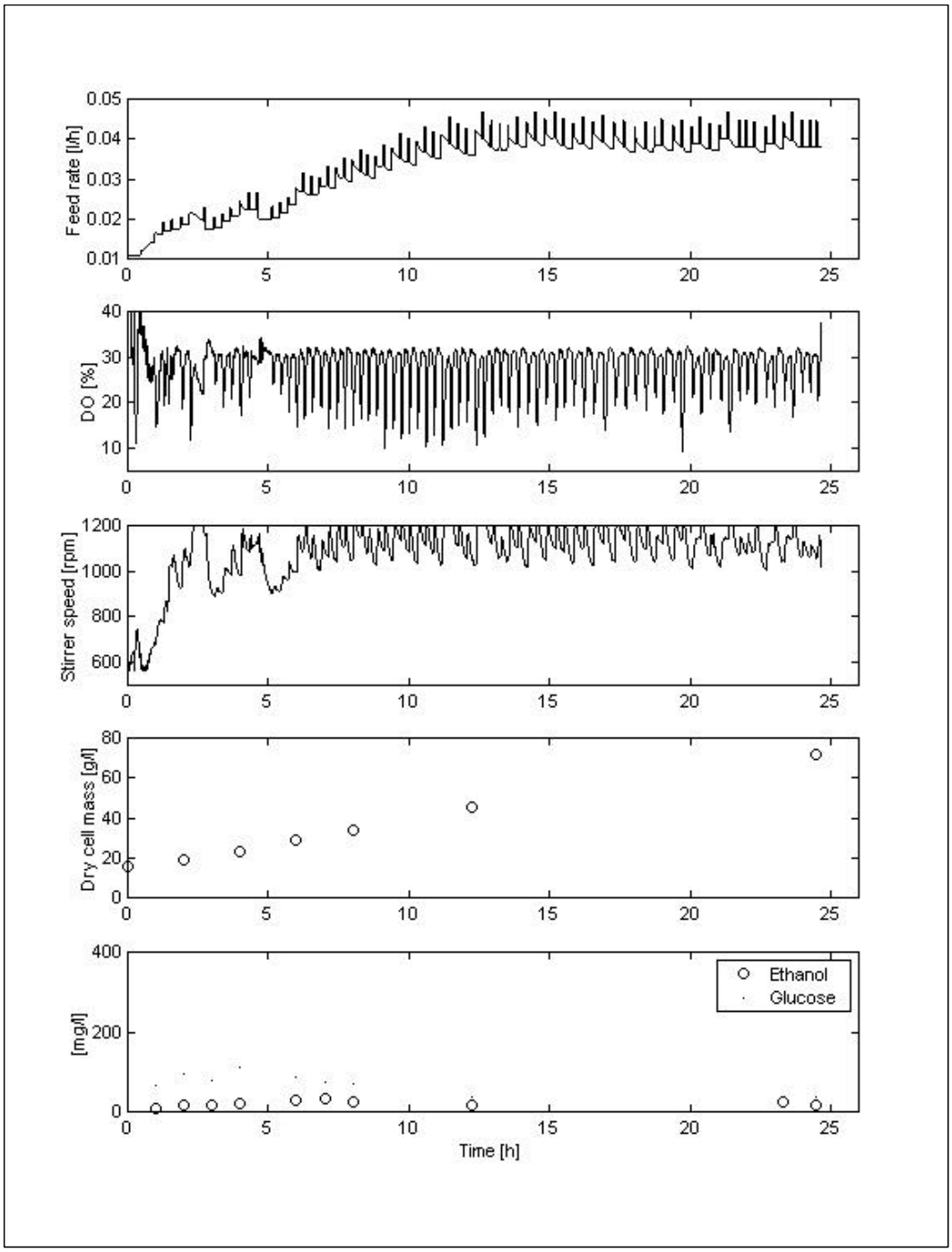

Figure 7.2 Fed-batch 16. Example of a fed-batch phase, where the “red” strain was used.

Approximate μ-values obtained during the exponential feeding period were:

μ ("blue" yeast)	0.10-0.12 h^{-1}
μ ("red" yeast)	0.08-0.11 h^{-1}

Although no standard deviation could be calculated, it appeared that μ_{blue} was slightly higher than μ_{red}.

$\mathbf{Y_{x/s}}$ ("blue" yeast)	0.46 ± 0.02 g/g
$\mathbf{Y_{x/s}}$ ("red" yeast)	0.48 ± 0.01 g/g

7.2.1 RQ-curve from a fed-batch cultivation with "blue" yeast

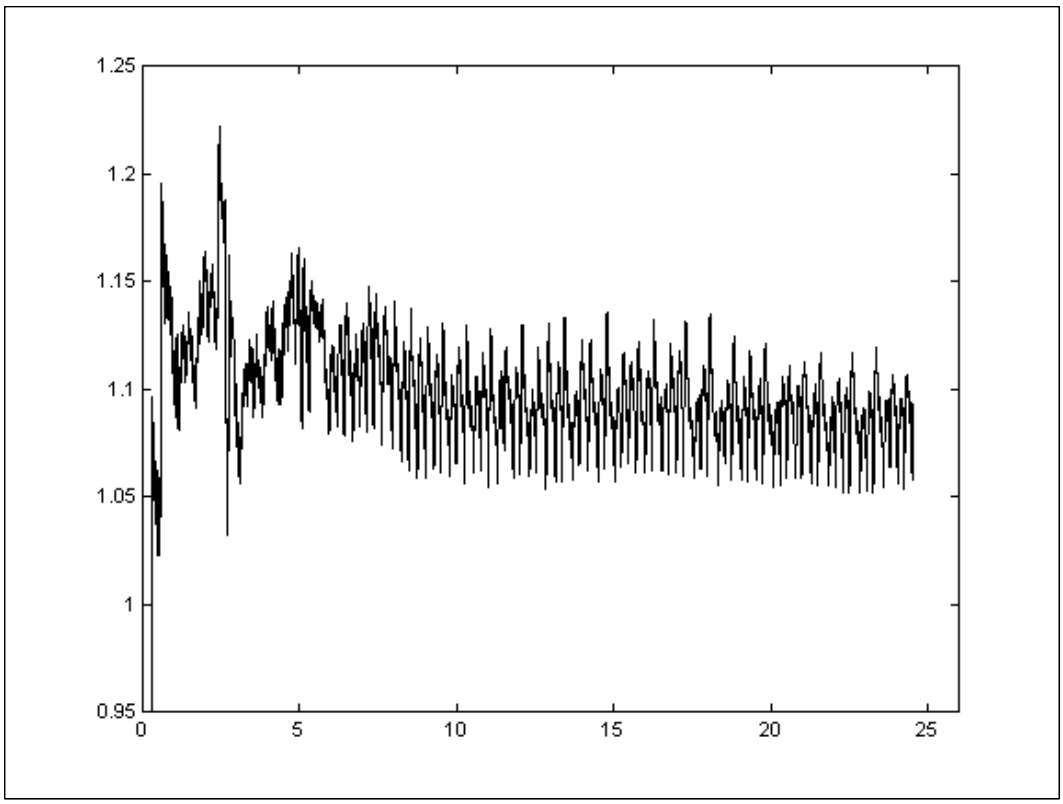

Figure 7.4 RQ during fed-batch 9

An RQ-curve for fed-batch 9 is shown in fig. 7.4. The RQ was close to 1.08 during the whole cultivation, which indicates that growth was respirative.

7.3 Probe responses

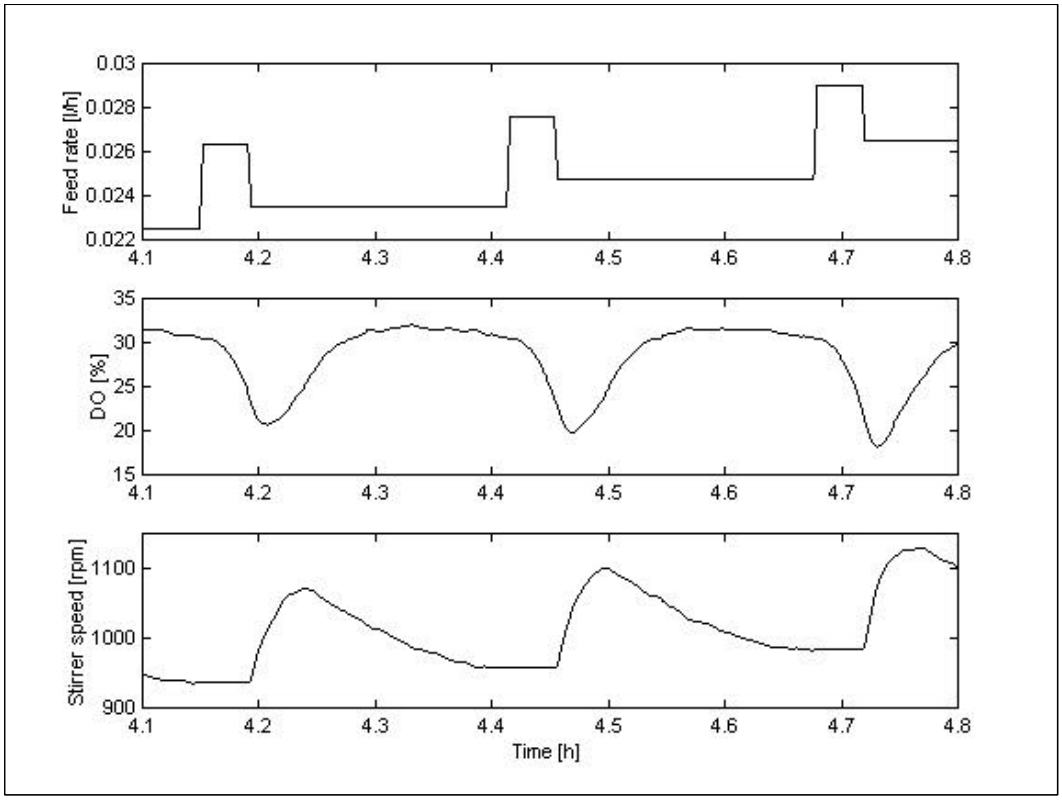

Figure 7.5 Magnification of some typical pulses from fed-batch 11. Their effects on DOT and stirrer speed are shown in the lower panels.

From figure 7.5 it can be seen that the effects from the pulses come with a certain delay. This delay is not included in the model, and has not been considered when the pulse length was chosen. It is obvious that the pulses do not fulfill the requirement for a pulse length greater than the overall time constant.
The bumpless transfer in the PID controller can be seen from the smooth transition of stirrer speed.

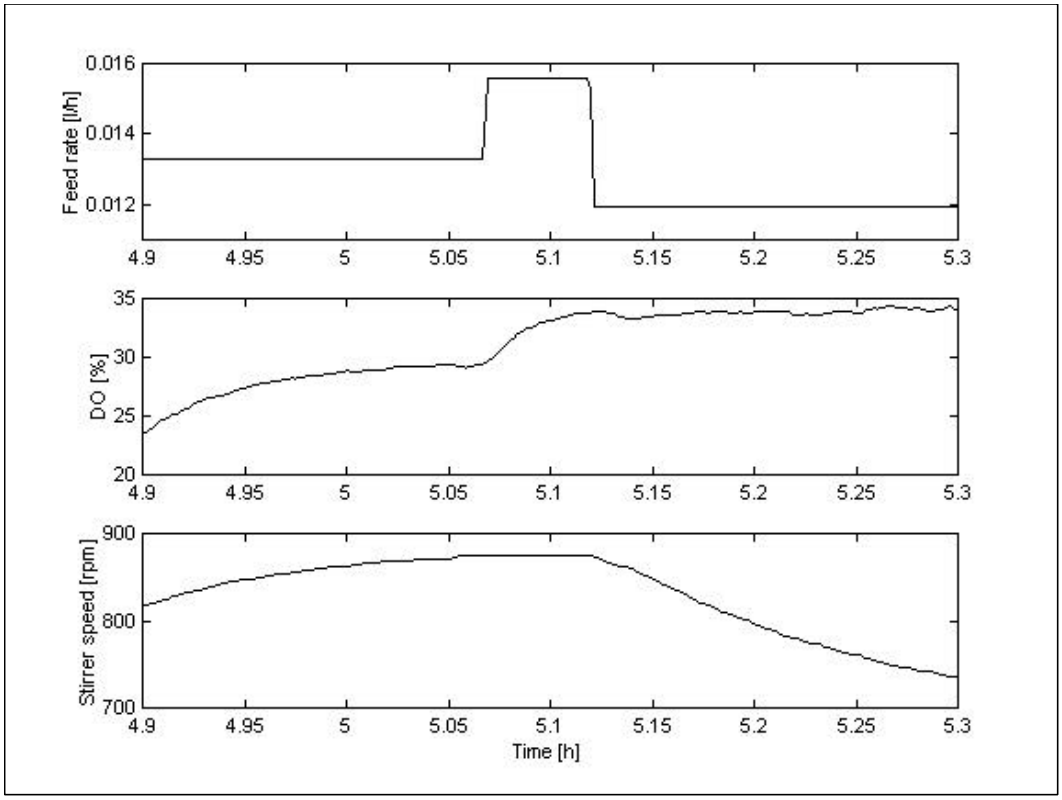

Figure 7.6 Magnification of one of the pulses in fed-batch 15 (“red” yeast).

On a few occasions, “negative” responses were obtained (fig. 7.6). At first we thought this phenomenon was caused by an occasional lowering of the specific oxygen uptake rate, when $q_s > q_s^{crit}$, due to a decrease in q_o caused by catabolite repression. But this was probably not the case. The time since the previous pulse had exceeded $T_{control}$ and thus the new pulse was triggered when the DOT entered the tolerance region (the DOT-curve had a positive slope when the pulse was issued). It can be concluded that whatever happened, it is not included in the model. Since the controller could not detect any response, the feed rate was lowered by 60% of the pulse.

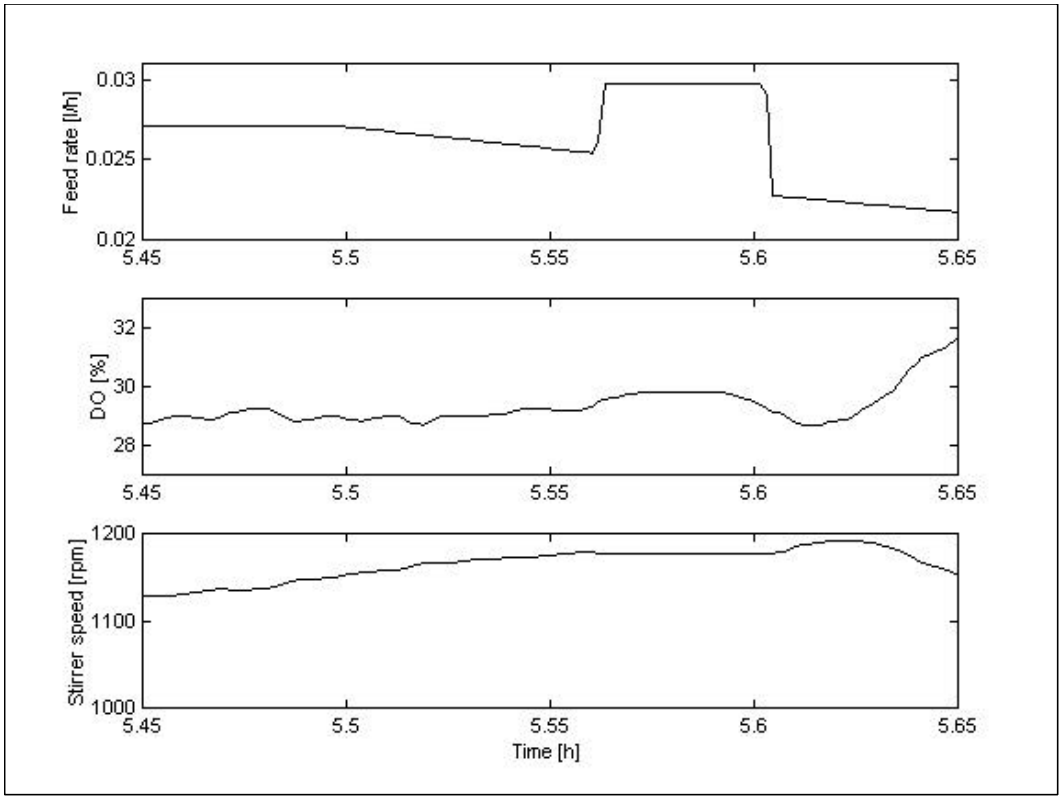

Figure 7.7 Magnification of one of the pulses in fed-batch 9 ("blue" yeast).

Figure 7.7 illustrates the safety net of the probing controller. The safety net is described in chapter 5.2.1. After 5.5 hours, the stirrer speed exceeded 1150 rpm, which was set to be the higher bound in the safety net. Since the stirrer was approaching its maximum speed, the feed rate had to be lowered to ensure a sufficient oxygen transfer to the reactor. A lowered feed rate made the cells consume less oxygen. The feed rate had to be lowered until the stirrer speed had gone below the allowed limit. Because the time since the last control period had exceeded $T_{control}$, the pulse was triggered when DOT came within the tolerance region.
There was no sufficient response to the pulse, which caused a lowered feed rate. Since the stirrer speed after the pulse was still too high, the continuous decrement in feed rate continued.

This behavior is not uncommon in the later phases of fed batches. See figure 7.2 and 7.3. These phases show an oscillating behavior where the controller is altering between increments and decrements of the feed rate.

Since the latter phase is a phase where the oxygen transfer forces the controller to lower the feed rate (q_s will be much lower than q_s^{crit}) and the oscillations do not occur due to jumps between the regions O_1 and O_3 in the stability plane, see fig. 5.1. The oscillations originate from the stirrer speed, which is oscillating between the higher and lower bounds, above 1150 rpm and below 1050 rpm. This causes the probing controller to apply adjustments of the feed rate according to the safety net rules.

7.4 Calculations of q_s

The μ-values obtained in the fed-batch cultivations with pulse were quite low compared to μ^{crit} obtained from literature, which was in the order of 0.25-0.30 h^{-1} (Enfors, et al. 1990). It seemed like q_s never reached q_s^{crit} during the exponential feeding phases. This was also shown by calculations:

$$q_s(t) \sim \frac{F(t) \cdot C_f}{X(t) \cdot V(t)}$$

Eq 7.1

(X also includes dead cells that do not consume glucose. It is assumed that the ratio between dead and living cells does not change significantly during the first 10 h)

X = cell mass concentration (g/l)
V = media volume
F = Feed rate (l/h)
C_f = glucose concentration in the feed solution (g/l)

Table 7.1 Values of q_s in fed-batch 17 ("blue" yeast). All of the values except for the last one were obtained during the exponential feeding period.

time (h)	q_s (g/g·h)
0	0.224
2	0.241
4	0.262
5,5	0.297
(12)	(0.160)

Table 7.2 Values of q_s in fed-batch 16 ("red" yeast). All of the values were obtained during the exponential feeding period. At 5 h there was a lowering of the feed rate, which can be seen in the decreased q_s at 6h.

time (h)	q_s (g/g·h)
0	0.177
2	0.233
4	0.246
6	0.217
8	0.234

q_s^{crit} = 0.51 g/g·h (Sonnleitner, et al. 1985)

7.5 Fed-batch cultivations without pulse feeding

Two fed-batch cultivations (A and B) with an exponential feeding profile, based on a μ of 0.12 h^{-1}, were also made to test if the μ-values obtained in the pulse feeding experiments would give purely respirative growth (fig 7.8, 7.9).

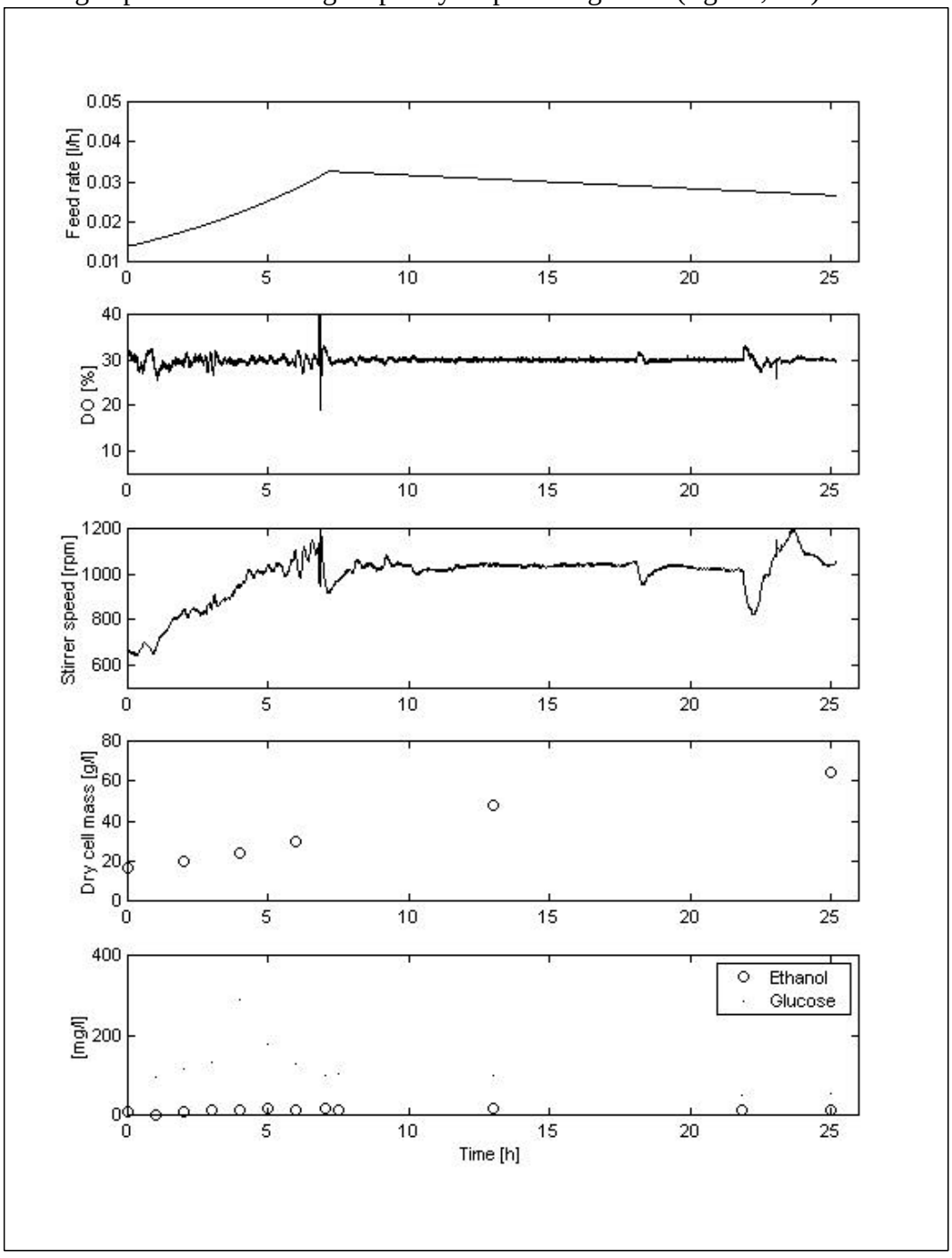

Figure 7.8 Fed-batch experiment with a predetermined feeding profile. The "blue" strain was used.

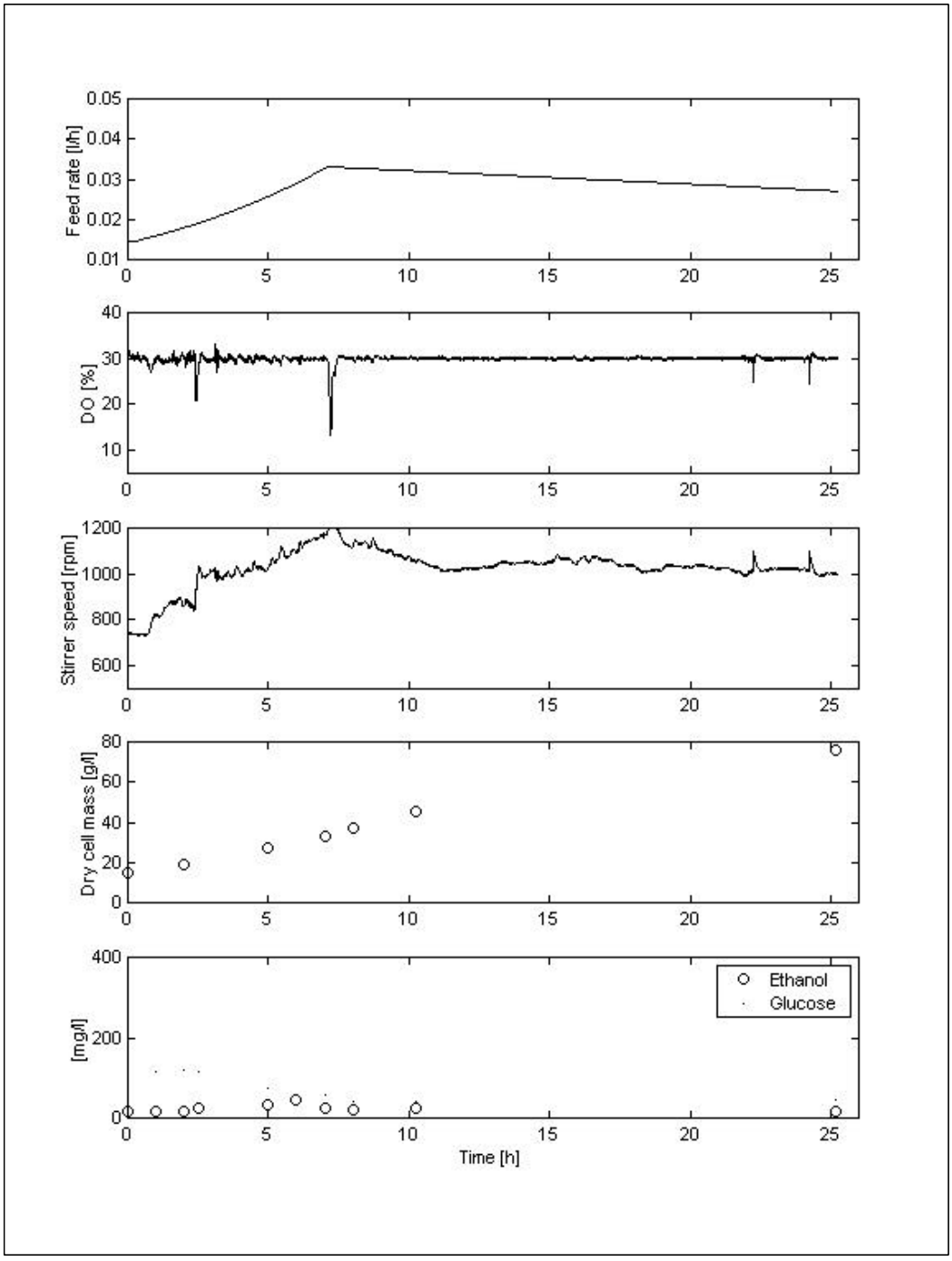

Figure 7.9 Fed-batch experiment with a predetermined feeding profile. The "red" strain was used.

$\mathbf{Y_{x/s}}$("blue")	0.48 g/g	**μ**("blue")	0.11 h^{-1}
$\mathbf{Y_{x/s}}$("red")	0.48 g/g	**μ**("red")	0.13 h^{-1}

(μ was calculated during the exponential feeding period)

The biomass yields shows that the growth was respirative, and the μ-values were as expected close to the exponent used in the predetermined feeding profile.

8 Discussion

8.1 Final conclusions

Our main objective was to keep the growth strictly respirative. To check this, different methods can be used. One of them is to measure the overall biomass yield on glucose, $Y_{x/s}$, during the cultivation, and compare it with yield values obtained during respiro–fermentative as well as purely respirative conditions.
In our case we saw that the yields for all the successful fed-batch cultivations were around 0.46 g/g for the blue yeast and around 0.48 g/g for the red yeast, compared to literature values that often are in the area of 0.49 g/g (respiratory growth) (Sonnleitner and Käppeli, 1985).
Given the uncertainties in the measurements, such as an unoptimized washing procedure, inaccuracy in the weighing procedure, etc, we can most likely conclude that the process was completely respirative.

The corresponding values of $Y_{x/s}$ during the batch cultivations with diauxic growth were about 0.30 g/g (they could have been slightly higher if ethanol loss in the exhaust gas could have been avoided), emphasizing that longer periods of respiro-fermentative glucose breakdown should have affected the fed-batch yields in a negative way.

The respiratory status was also confirmed by ethanol and glucose concentration measurements (see Appendix C). As observed the ethanol concentrations did only exceed 50 mg/l a handful of times, which again underline that our fed-batch cultivations were totally respirative.
The accuracy of these measurements can be discussed. Due to practical reasons the cells could not be filtered away during sampling. Even if the time passed between the filling of the tubes, and the starting of the centrifuge, never exceeded 20 s, a considerable amount of the substrate to be measured (ethanol and glucose) was consumed by the cells. If hydrochlorous acid had been added to the Eppendorf tubes before sampling this could have been avoided.
The obtained values were therefore only interesting in order to find those occasions during the experiment, where the glucose or ethanol concentrations reached considerable amounts (several hundred mg/l).

Another useful tool when studying the respirative status during cultivation is by looking at the RQ-values (Appendix J). At respiratory growth RQ is close to 1.08 (Axelsson, 1989). During fed-batch 9 the gas analyzer was connected, giving us an RQ-profile for the fed-batch cultivation (fig 7.4).
The RQ was always close to 1.08, though a bit higher during the first 7 h, when the feed rate was increasing exponentially. The result just emphasizes that growth during the fed-batch cultivation was respirative. The RQ was above 1.2 for a short period at 2h. It is interesting to notice that during this period of time the feed rate was decreased two times in a row.

For the 7 successful fed batches, the μ-values were calculated. In order to get the maximum growth rate, the cell mass concentrations used were those of the exponential feeding period. The mean value was ca 0.12 h^{-1} for the blue yeast and ca 0.10 h^{-1} for the red, which is low compared to 0.25-0.30 h^{-1} for μ^{crit} (Enfors et al., 1990).
It seemed like our feed rate had not increased enough for q_s to reach q_s^{crit}, and even if q_s was approaching q_s^{crit}, the regulator was forced to decrease the feed rate due to saturation in the oxygen transfer rate, before convergence was reached.
This was observed when q_s was calculated for two fed-batch cultivations (chapter 7.4, table 7.1, 7.2). In fed-batch 17 ("blue" yeast) the q_s increased during the exponential phase, but didn't reach q_s^{crit}. During fed-batch 16 ("red" yeast) q_s decreased between 4h and 6h due to a lowering in feed rate. No convergence between q_s and q_s^{crit} was seen here either.

This conclusion was further proven when the results from the two fed batches, without pulse feeding, were examined. The feed rate increased in accordance with a predetermined profile based on a μ-value of 0.12 h^{-1} and a F_0 of 0.014 l/h. When DOT no longer could be held at 30 % by the PID controller, a slowly decreasing feed profile was applied. This trend was based on an average of the pulse feeding experiments.
If q_s reached or exceeded q_s^{crit} for longer periods in the pulse feeding experiments the signs of over-flow metabolism would have been even more obvious in the non-pulse experiments (no decrements could be done). However, none of these signs were observed, in the non-pulse experiments, for neither the red nor the blue yeast. The yields were ca 0.48 g/g for the blue and the red yeast, the ethanol values less than 50 mg/l and the RQ was close to 1.08 during the two fed-batches.

According to these results one would think that there had been no lowerings of the feed rate during the fed-batch cultivations. This is actually not the case; in some of the fed-batch experiments there were one or two decreases in feed rate during the exponential phase. It is obvious that during, and shortly after, these pulses no decrease in DOT could be found and in connection to some of these lowerings high ethanol concentrations were found (examples: at 5:30 h in fed batch 15 and at 2 h in fed batch 8).

During the feed pulses before decrements, the corresponding q_s-values never exceeded 0.40 g/g·h, compared to the theoretical q_s^{crit} of approx. 0.51 g/g·h (Sonnleinter and Käppeli, 1986.). It seemed like q_s^{crit} varied during a fed batch experiments and we didn't have any direct explanation. According to different sources, yeast cells grown on a defined media at low dilution rates (ca 0.10 h^{-1}) tend to adjust the glucose uptake rate in a cell-cycle dependant way, which leads to a non-constant q_s^{crit} (Birol et al., 1999).
The reason for these oscillations is not well known to us but they could be due to different stages in the glycogen / trehalose metabolism. At certain times in the cell cycle trehalose is broken down, and if the cell culture is synchronized (the main part of the cells are in the same phase of the cell-cycle) during this short phase of

over-flow metabolism, it results in an increased ethanol concentration in the medium (up to several hundred mg/l).
This problem of synchronization does not occur in cultures grown on complex media like molasses, and can be avoided in glucose fed cultures by adding a small amount of ethanol. (Fiechter and Seghessi, 1991).

8.2 Comparison between the two yeast strains

As mentioned before two commercial strains of Baker's yeast, obtained from Jästbolaget AB, Sweden, were used. The so-called blue strain (for ordinary doughs) and the so-called red strain (for sweet doughs).
By studying the batch phases some interesting details could be seen (chapter 7.1, fig 7.1). At the end of phase 1 (glucose consumption), the trend differed, perhaps due to differences in the acetate metabolism. Another striking difference was that the ethanol consumption phase was longer for the "red" yeast. This could e.g. be explained by limited respiratory capacity for the "red" yeast compared to the "blue".

Neither the "red" nor the "blue" yeast responded immediately to a feed pulse. There was at least a 40 s delay for the "blue" yeast and a 50 s delay for the "red" yeast, before the decrease in DOT started.
This can partly be explained by effects from the delay time of the oxygen probe and the feed pump. Some experiments to find out the delay time that could be referred to the equipment were made, and it was found out that it was maximum 20 s. The conclusion was that a large part was due to some biochemical feature of the yeast cells.

The most striking difference between "red" and "blue" yeast appeared when the first pulse feeding experiment with the "red" yeast was started (fig 8.1). The same pulse length (150 s), which had worked for the "blue" yeast, was used. The increases in feed rate were just tiny and were therefore often followed by feed rate decrements.
When the pulse length was prolonged from 150 s to 180 s, the increase in feed rate accelerated (observe the larger decreases in DOT during the last 4 h). During the next fed-batch cultivation with red yeast (fed batch 15), the pulse length was 200 s and the feed profile increased in much the same way as for the "blue" yeast.

The small difference in delay time ca 10 s can not explain why the pulse time had to be that much longer for the "red" yeast.
Perhaps the K_s of the "red" yeast is larger than for the "blue". This would lead to a slower increase in q_s, when a pulse is applied. Maybe the dynamics of the specific glucose uptake rate for the two strains can't be properly described by the Monod kinetics. The relation between S and q_s could be more like an s-shaped curve (fig 8.2), with an initial "horizontal" phase, which would be more pronounced for the "red" yeast. It should be emphasized that these explanations are highly speculative.

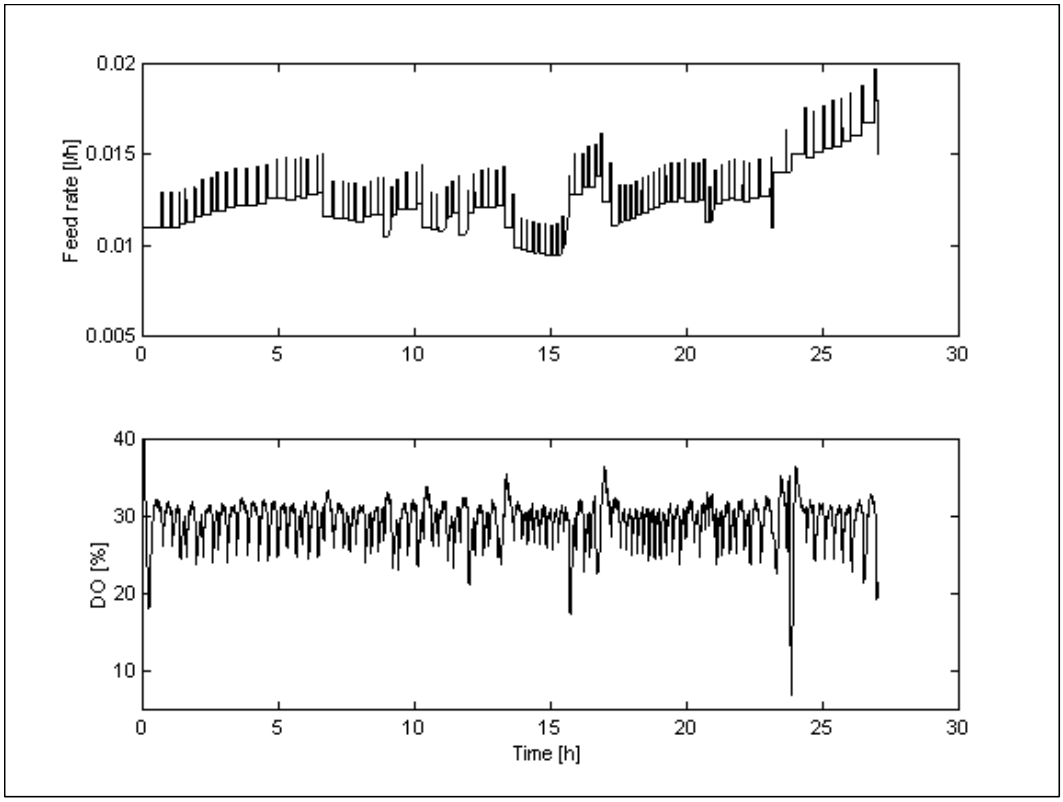

Figure 8.1 Feed rate and DOT in fed-batch 14. After 24 h the pulse length was increased from 150 s to 180 s.

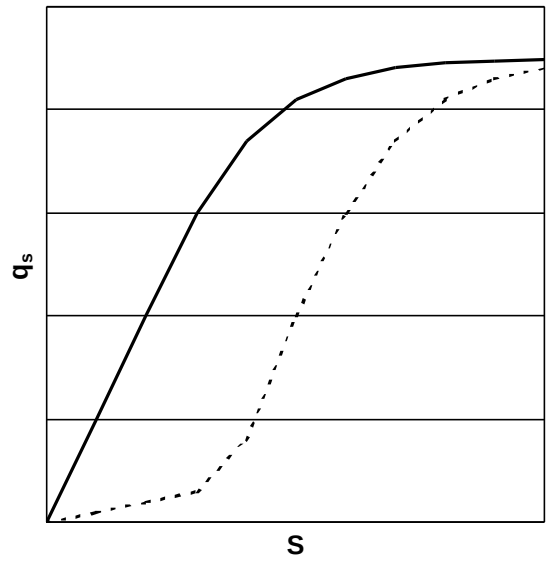

Figure 8.2 A simple Monod relation between q_s and S (-) compared to a relation with an initial "horizontal" phase (---).

8.3 Improvements and future work

Improvements to the control strategy should be made before the method can be used in a successful and reproducible way. Primarily the productivity has to be improved, in other words; μ has to be increased.
It is therefore essential to reach convergence between q_s and q_s^{crit} as fast as possible without over-flow metabolism.

Choosing the correct pulse time is essential for the performance of the pulse feeding strategy. The difficulties in reaching q_s^{crit} could be due to a too short pulse length. The ß parameter from the model in chapter 3 will then be smaller than 0.63, and the pulse responses will not be as large as expected.
Some experiments (where the step response to an increase in F was observed) as well as theoretical calculations seemed to give pulse times close to those we used.
A too large increase of the pulse length on the other hand can create problems such as impaired stability, too long control periods (affects the ability to reach q_s^{crit} in a negative way), extensive ethanol accumulation in the media, etc.

It could prove successful to increase the initial feed rate (a higher initial q_s), but it would have drawbacks too. In the beginning of the fed-batch the medium volume is small. As a result the K_La-value is also relatively small, which could lead to a saturation of the oxygen transfer (N reaches 1200 rpm) relatively early in the exponential phase.

A third measure could be to decrease the initial cell mass concentration to about 4-5 g/l (see the simulations in chapter 5.5, figure 5.4). This would prolong the exponential phase and thereby increase the chance to reach convergence. The ratio between initial feed rate and initial cell mass concentration might also be increased without risking oxygen transfer saturation early during the fed-batch.
In our case the last suggestion wasn't applicable because of the poor performance by the feed pump at low feed rates.

The oxygen controller does not work as well as it could. The latter phases of most fed-batch cultivations show an oscillatory behavior that could possibly be avoided with a better tuned PI controller. Another alternative is to have a self-tuning controller instead of a PI controller with gain scheduling. The controller would then estimate parameters that are correct with respect to the reactor volume as well as the stirrer speed.

Over-flow metabolism in the latter phase does not occur (high cell mass concentration but an almost constant feed rate). The pulses could therefore be removed when the oxygen transfer reaches it's maximum. The safety net measures would still be useful for manipulating the feed rate to ensure that the oxygen transfer is sufficient.

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We are also grateful for the help we received from Anneli Nilsson at Chemical Engineering II, LTH.

A. Fed-batch experiments

A short description of the fed-batch cultivations

Fed-batch 8

Yeast used:	"Blue" yeast
Glucose concentration in the feed solution:	400 g/l
Initial feed rate, F_0:	0.011 l/h
Pulse length	150 s
Maximal feed rate increase after a pulse:	No limitations
Maximal feed rate lowering after a pulse:	100 % of a pulse height
The slope factor in the safety net:	1

Fed-batch 9

Yeast used:	"Blue" yeast
Glucose concentration in the feed solution:	400 g/l
Initial feed rate, F_0:	0.011 l/h
Pulse length	150 s
Maximal feed rate increase after a pulse:	No limitations
Maximal feed rate lowering after a pulse:	60 % of a pulse height
The slope factor in the safety net:	0.5

Fed-batch 10

Yeast used:	"Blue" yeast
Glucose concentration in the feed solution:	400 g/l
Initial feed rate, F_0:	0.011 l/h
Pulse length	150 s
Maximal feed rate increase after a pulse:	No limitations
Maximal feed rate lowering after a pulse:	60 % of a pulse height
The slope factor in the safety net:	0.33

Fed-batch 11

Yeast used:	"Blue" yeast
Glucose concentration in the feed solution:	400 g/l
Initial feed rate, F_0:	0.013 l/h
Pulse length	150 s
Maximal feed rate increase after a pulse:	One pulse height
Maximal feed rate lowering after a pulse:	60 % of a pulse height
The slope factor in the safety net:	0.33

Fed-batch 13

Yeast used:	"Red" yeast
Glucose concentration in the feed solution:	400 g/l
Initial feed rate, F_0:	0.011 l/h
Pulse length	150s
Maximal feed rate increase after a pulse:	One pulse height
Maximal feed rate lowering after a pulse:	60 % of a pulse height
The slope factor in the safety net:	0.33

(No significant increase in feed rate during the fed-batch cultivation)

Fed-batch 14

Yeast used:	"Red" yeast
Glucose concentration in the feed solution:	325 g/l
Initial feed rate, F_0:	0.011 l/h
Pulse length	150 s the first 24 h then 180 s
Maximal feed rate increase after a pulse:	One pulse height
Maximal feed rate lowering after a pulse:	60 % of a pulse height
The slope factor in the safety net:	0.33

(No significant increase in feed rate during the fed-batch cultivation)

Fed-batch 15

Yeast used:	"Red" yeast
Glucose concentration in the feed solution:	325 g/l
Initial feed rate, F_0:	0.011 l/h
Pulse length	180 s the first 5h then 200 s
Maximal feed rate increase after a pulse:	One pulse height
Maximal feed rate lowering after a pulse:	60 % of a pulse height
The slope factor in the safety net:	0.33

Fed-batch 16

Yeast used:	"Red" yeast
Glucose concentration in the feed solution:	325 g/l
Initial feed rate, F_0:	0.011 l/h
Pulse length	200 s
Maximal feed rate increase after a pulse:	One pulse height
Maximal feed rate lowering after a pulse:	60 % of a pulse height
The slope factor in the safety net:	0.33

Fed-batch 17

Yeast used:	"Blue" yeast
Glucose concentration in the feed solution:	400 g/l
Initial feed rate, F_0:	0.013 l/h
Pulse length	150 s
Maximal feed rate increase after a pulse:	One pulse height
Maximal feed rate lowering after a pulse:	60 % of a pulse height
The slope factor in the safety net:	0.33

Fed-batch 18

Yeast used:	"Blue" yeast
Glucose concentration in the feed solution:	400 g/l
Initial feed rate, F_0:	0.013 l/h
Pulse length	150 s
Maximal feed rate increase after a pulse:	One pulse height
Maximal feed rate lowering after a pulse:	60 % of a pulse height
The slope factor in the safety net:	0.33

(Due to computer problems no values were logged.)

Fed-batch A (predetermined feed profile)

Yeast used:	"Blue" yeast
Glucose concentration in the feed solution:	400 g/l
Initial feed rate, F_0:	0.014 l/h
Exponential feed profile:	$0.014 \cdot e^{0.12\,t}$ (l/h)
Decreasing linear feed profile:	$F_1^* - (9.26 \cdot 10^{-8} \cdot t)$ (l/h)

Fed-batch B (predetermined feed profile)

Yeast used:	"Red" yeast
Glucose concentration in the feed solution:	400 g/l
Initial feed rate, F_0:	0.0145 l/h
Exponential feed profile:	$0.0145 \cdot e^{0.12\,t}$ (l/h)
Decreasing linear feed profile:	$F_1^* - (9.26 \cdot 10^{-8} \cdot t)$ (l/h)

* The feed rate obtained when N had reached 1200 rpm.

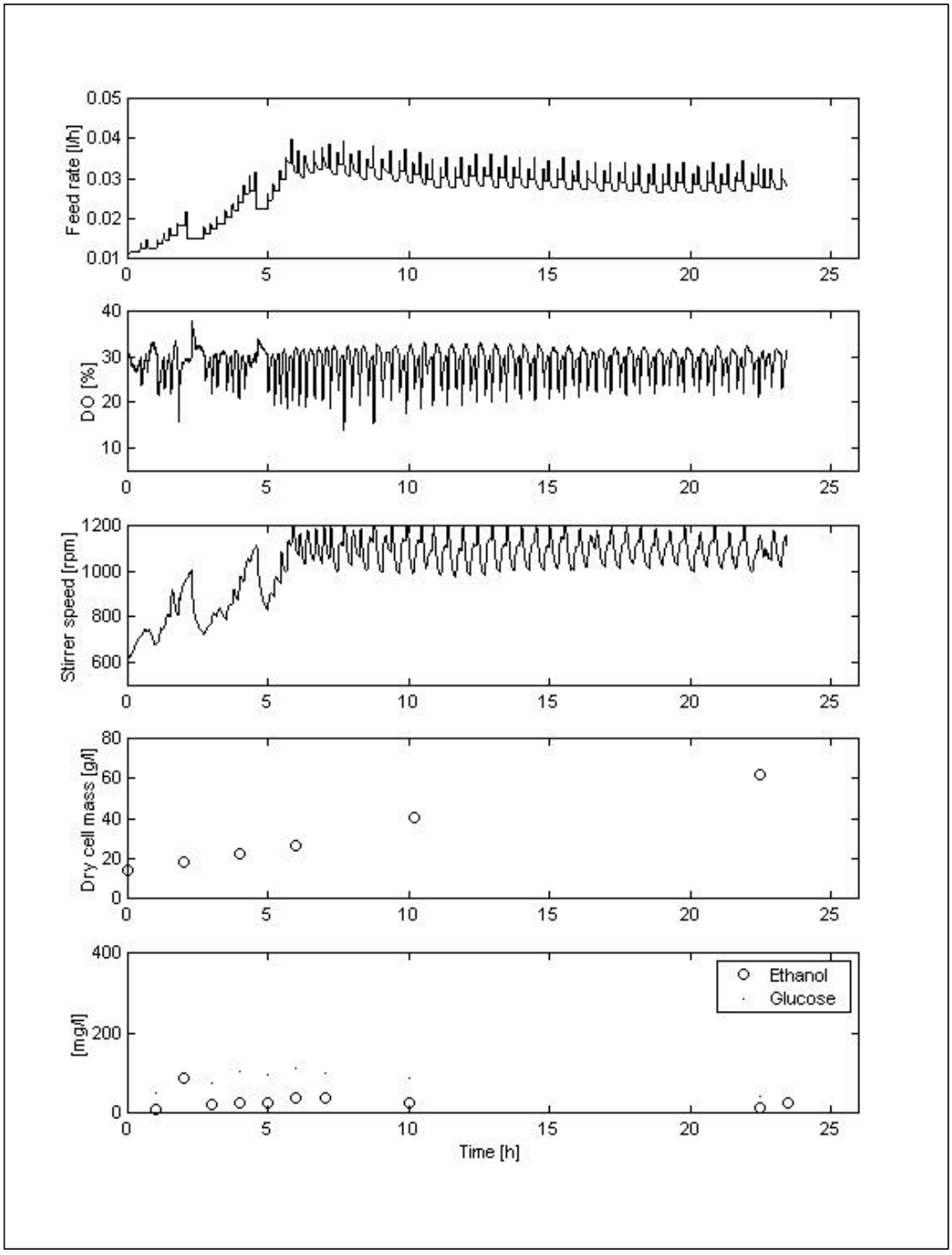


Figure A.1 Fed-batch 8

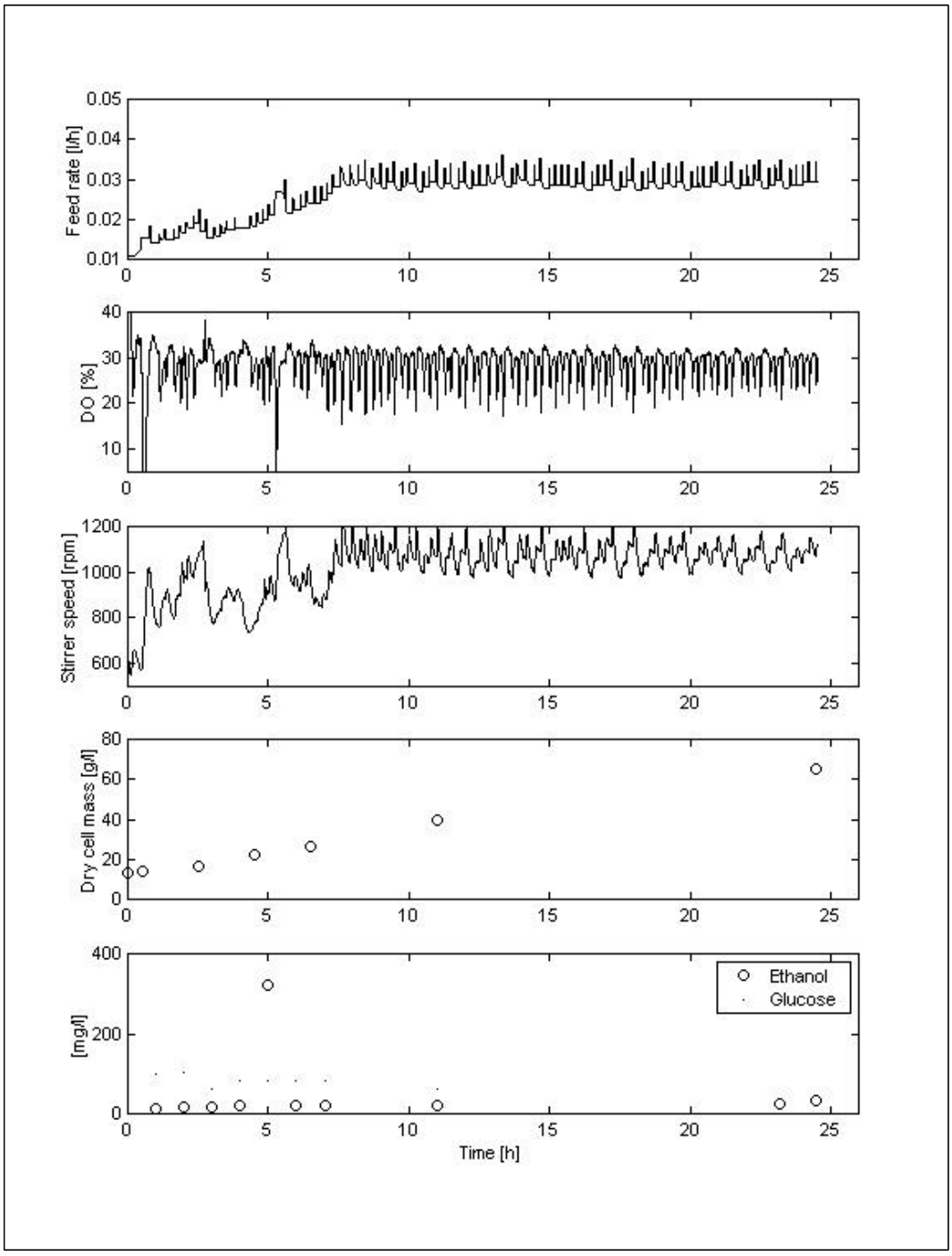


Figure A.2 Fed-batch 9

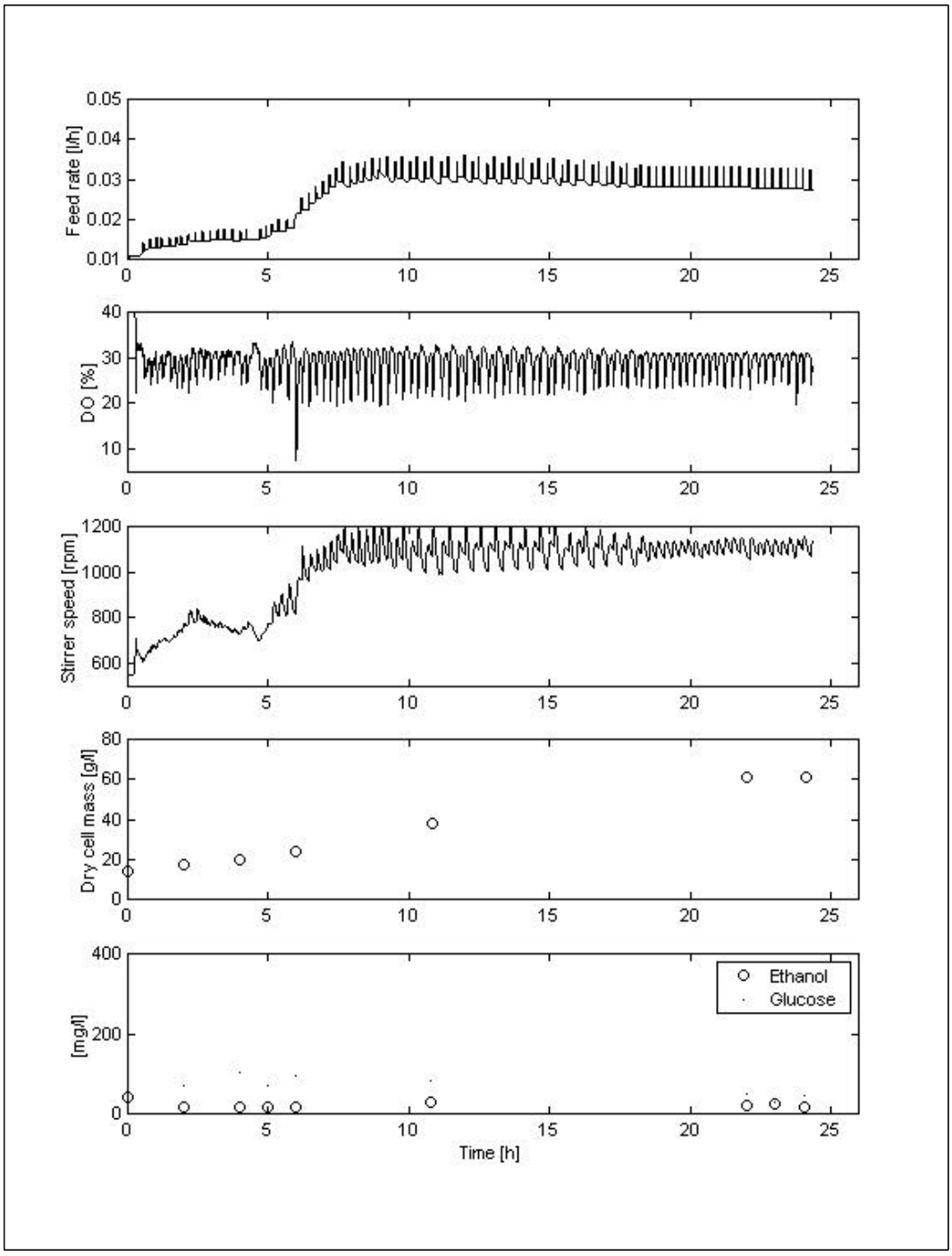


Figure A.3 Fed-batch 10

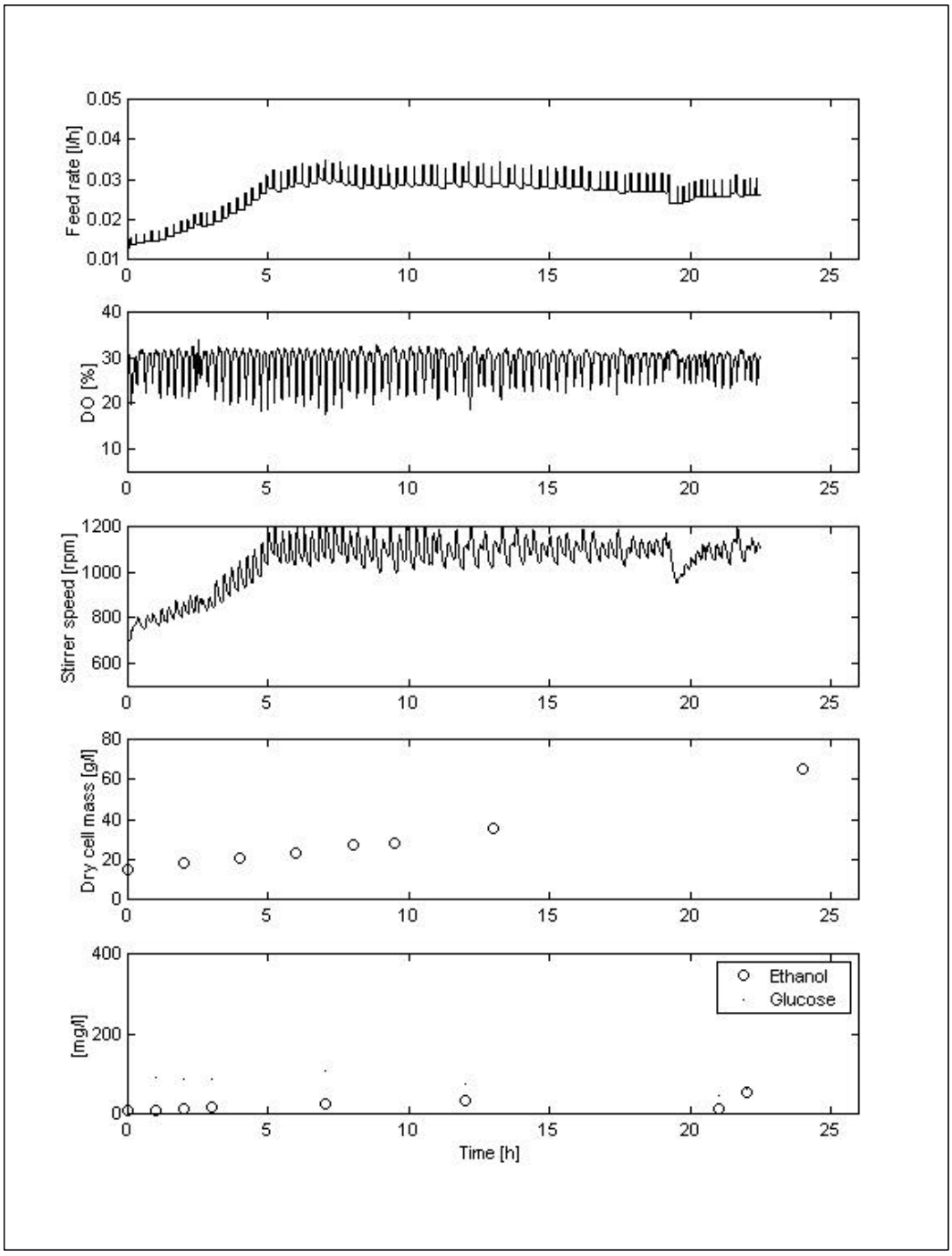


Figure A.4 Fed-batch 11

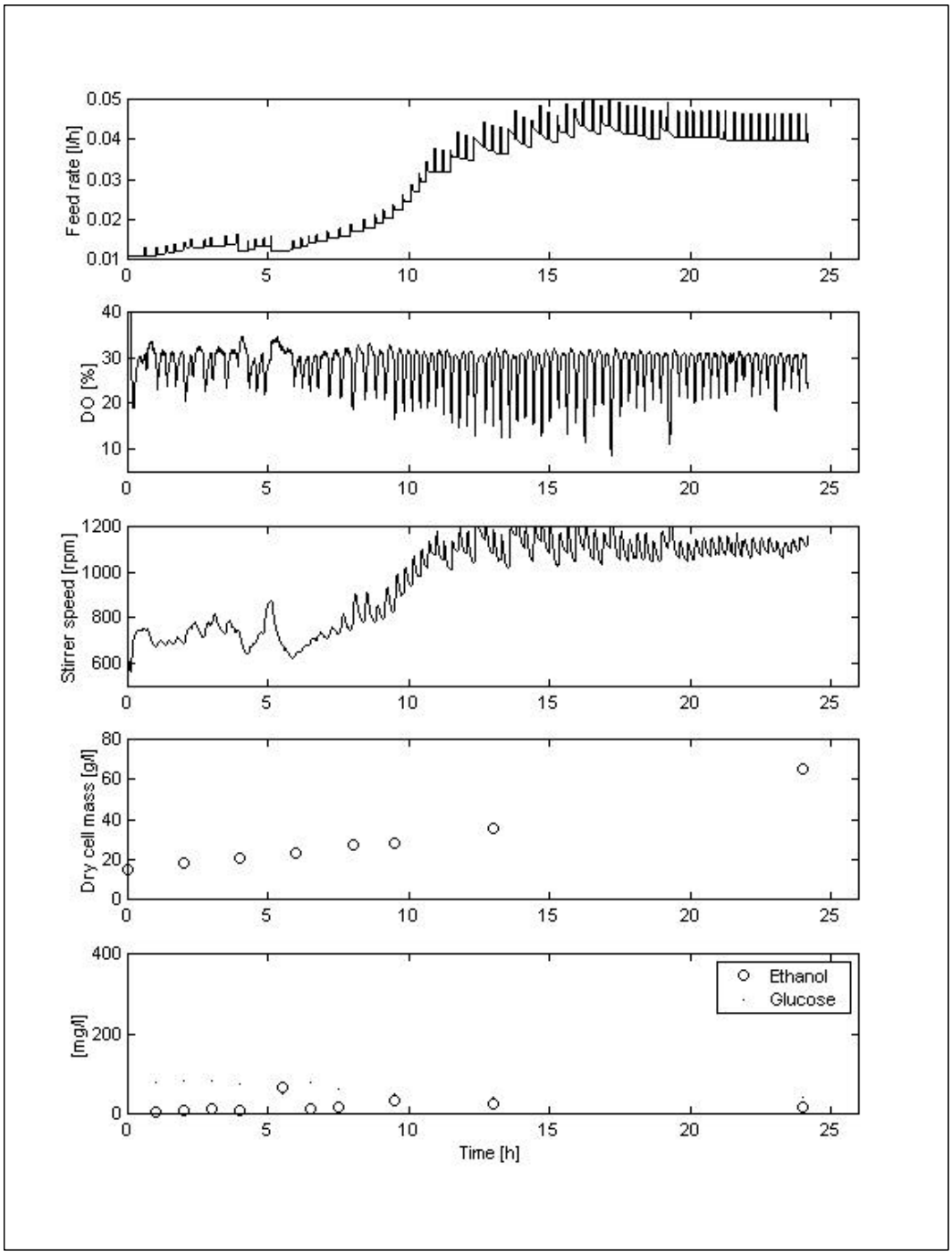


Figure A.5 Fed batch-15

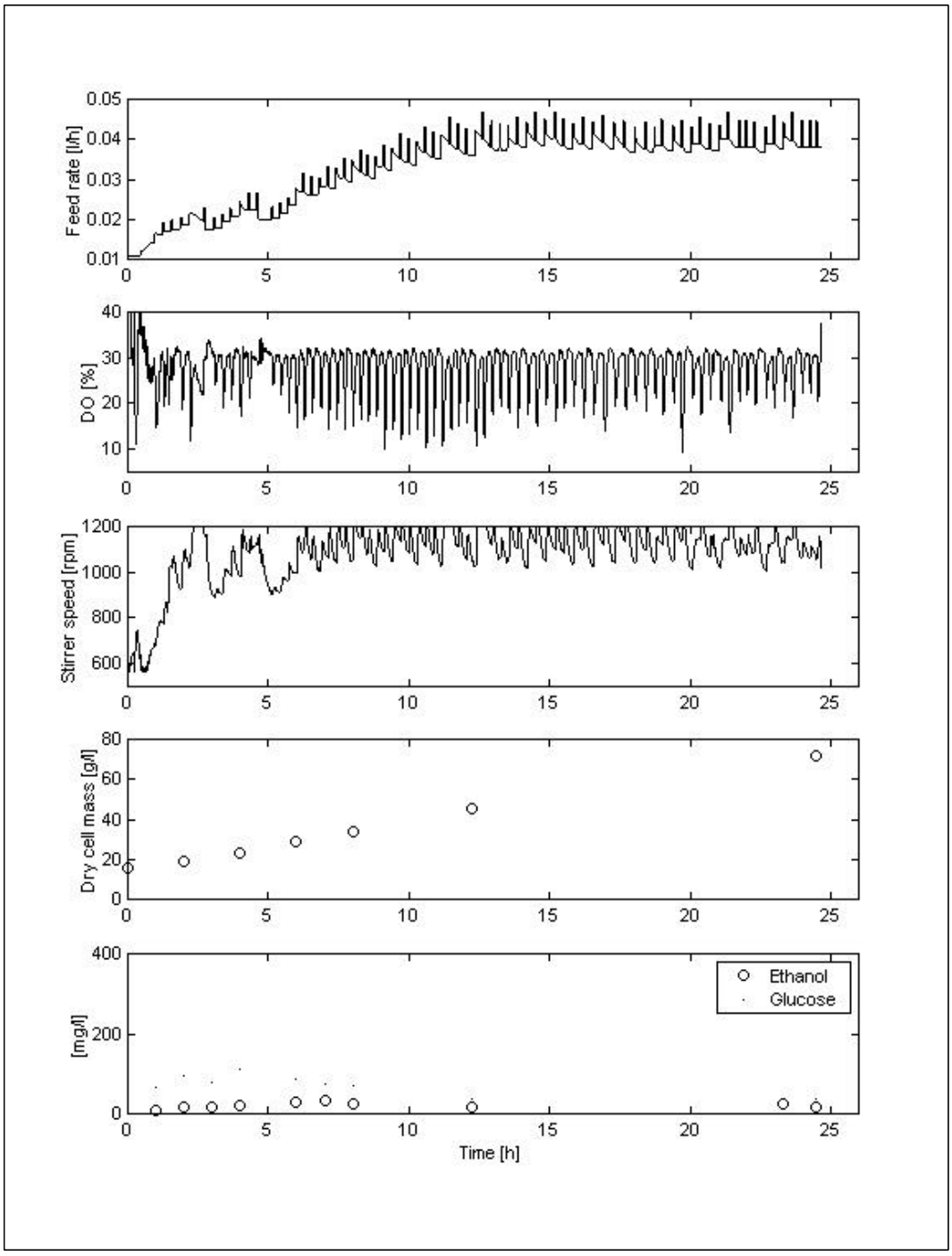


Figure A.6 Fed-batch 16

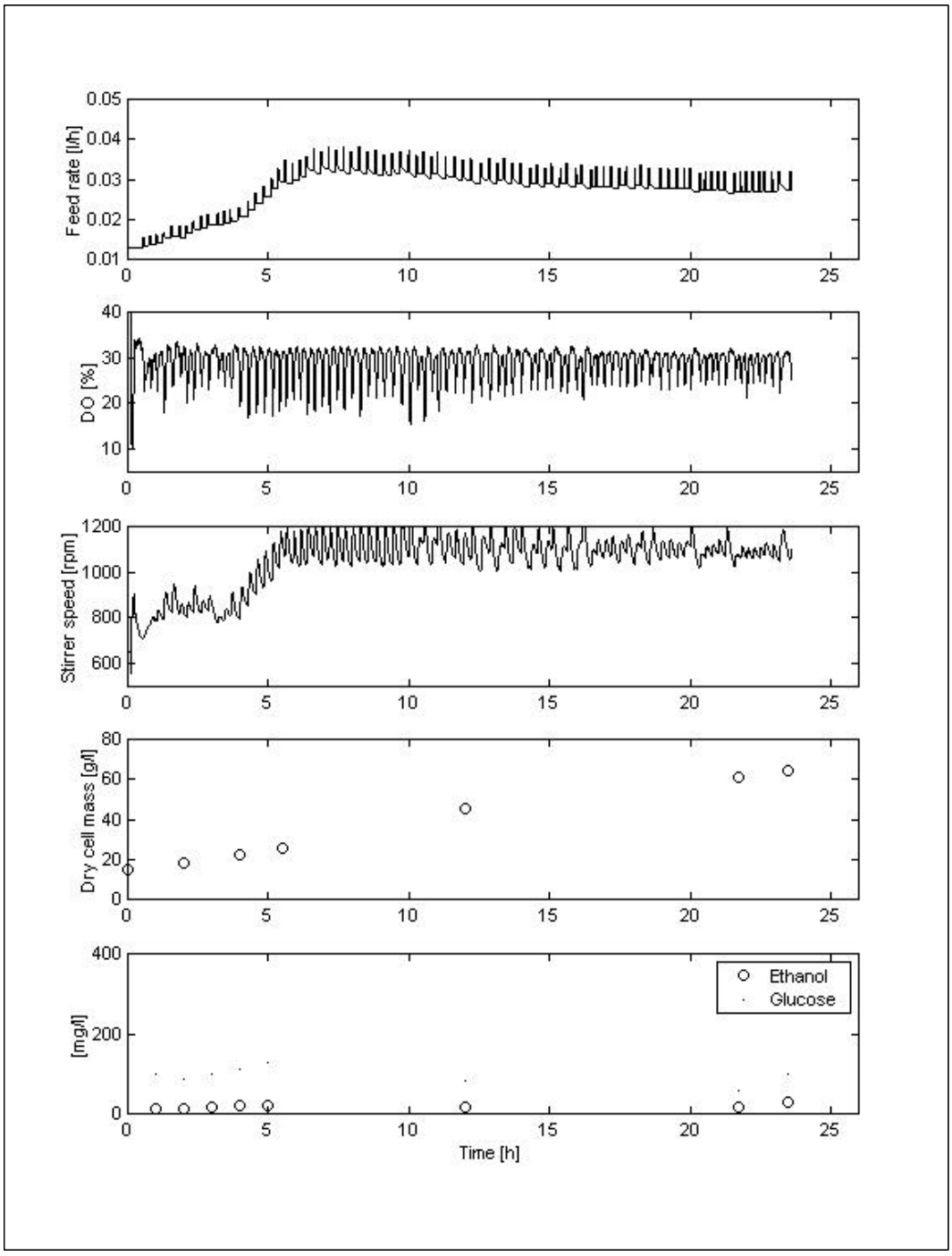


Figure A.7 Fed-batch 17

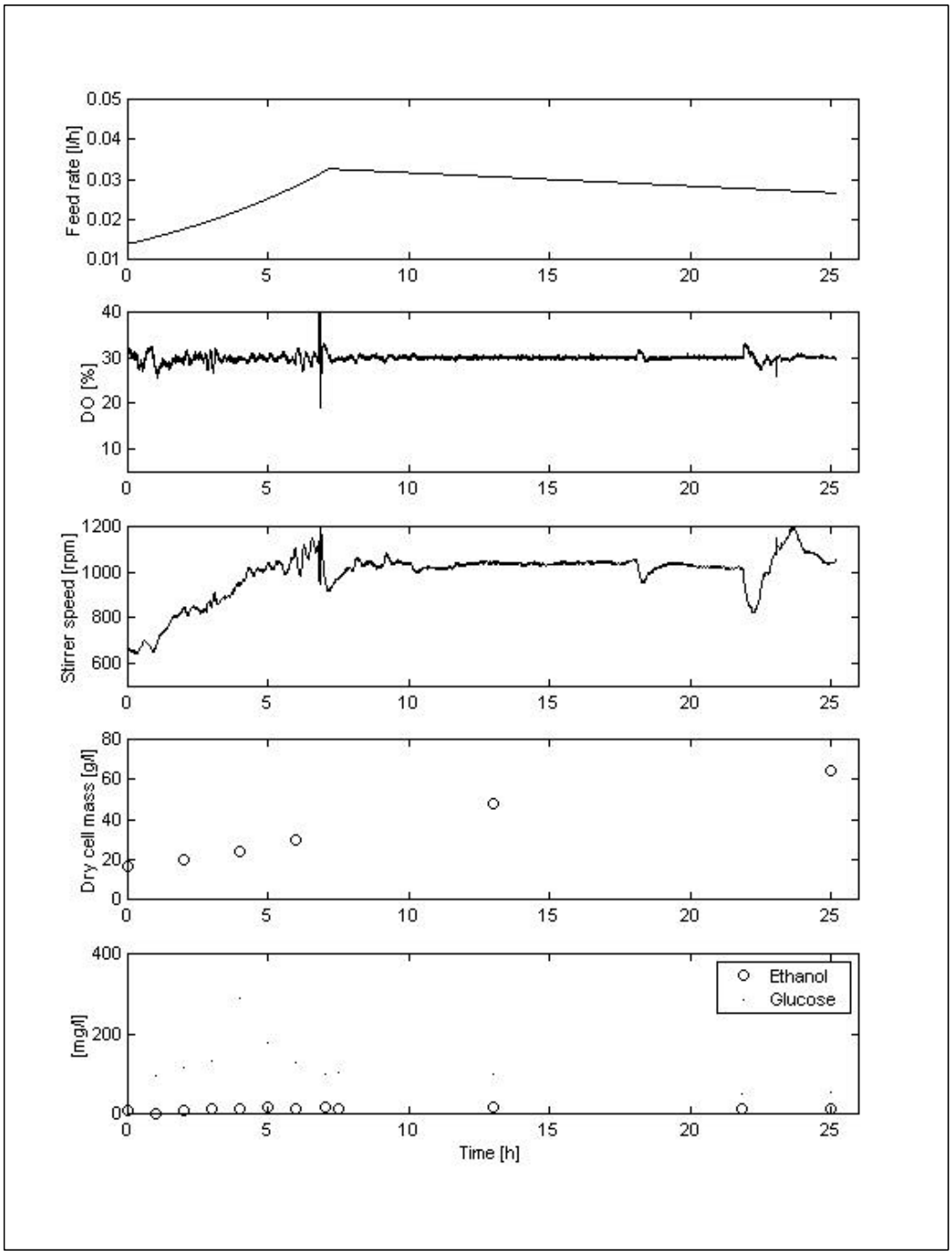


Figure A.8 Fed-batch A

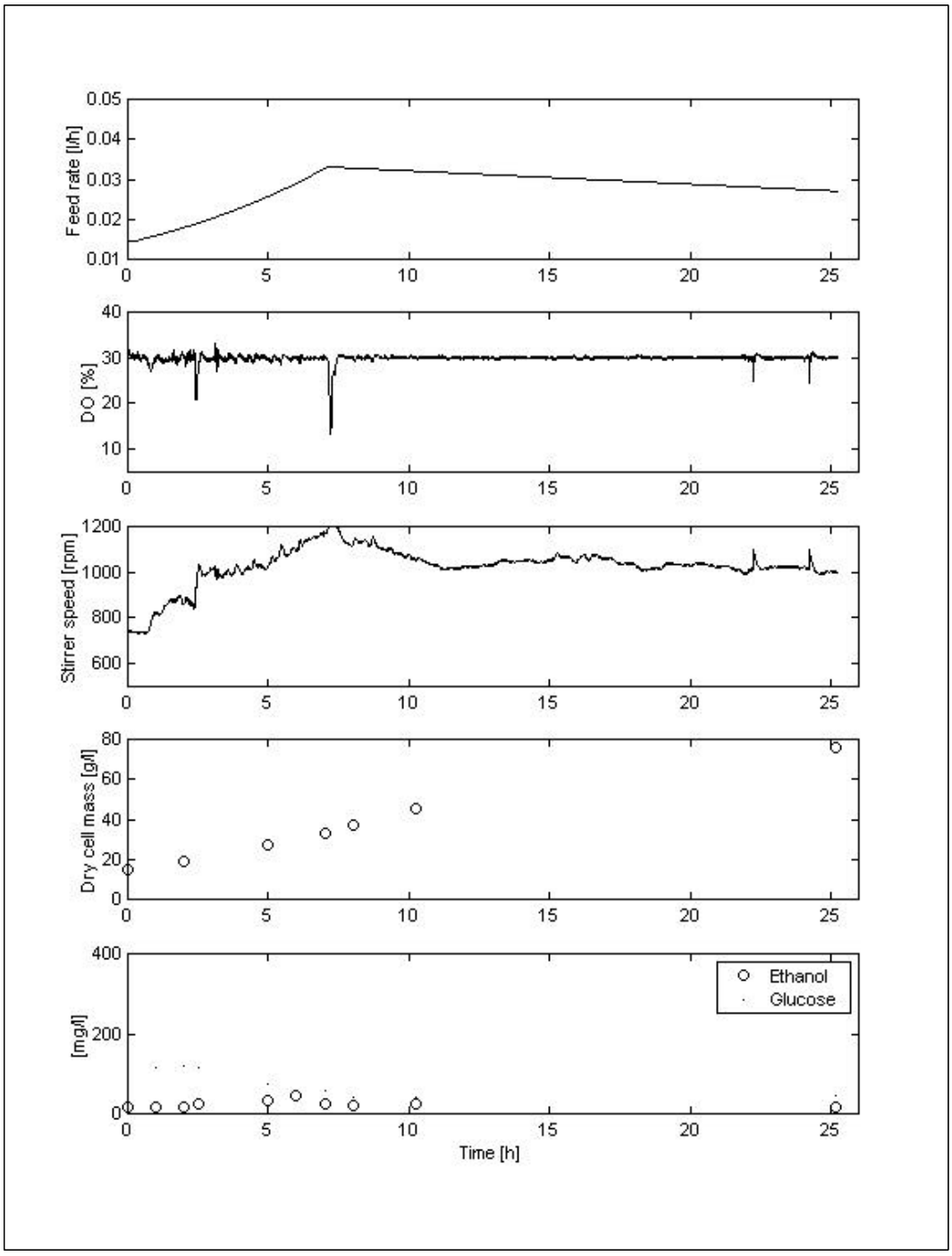


Figure A.9 Fed-batch B

B. Measured cell mass concentrations

Cell mass concentrations

Table B.1 X in fed-batch 8 ("blue" yeast)

time (h)	**X** (g/l)
0	13,8
2	18,2
4	21,9
6	26,7
10,16	40,7
22,5	61,5

Table B.2 X in fed-batch 9 ("blue" yeast)

time (h)	**X** (g/l)
0	13,1
0,5	13,8
2,5	16,6
4,5	21,9
6,5	26,4
11	39,8
24,5	65,5

Table B.3 X in fed-batch 10 ("blue" yeast)

time (h)	**X** (g/l)
0	14,2
2	17
4	20,2
6	24
10,83	37,9
22	60,7
24,1	61,3

Table B.4 X in fed-batch 11 ("blue" yeast)

time (h)	**X** (g/l)
0	15
2	18,2
3,5	21,2
7	31,2
12	46,5
21	59,7
22,5	58,1

Table B.5 X in fed-batch 17 ("blue" yeast)

time (h)	**X** (g/l)
0	14,8
2	17,8
4	22
5,5	25,7
12	45,6
21,75	60,8
23,5	64,4

Table B.6 X in fed-batch 15 ("red" yeast)

time (h)	**X** (g/l)
0	14,9
2	18,1
4	20,4
6	23,2
8	27,4
9,5	28,3
13	35,7
24	65,5

Table B.7 X in fed-batch 16 (“red” yeast)

time (h)	**X** (g/l)
0	15,6
2	19,3
4	23,4
6	28,8
8	34,2
12,25	45,7
24,5	71,9

Table B.8 X in fed-batch A (“blue” yeast)

time (h)	**X** (g/l)
0	16.1
2	19.5
4	23.6
6	29.4
13	47.7
25	64.5

Table B.9 X in fed-batch B (“red” yeast)

time (h)	**X** (g/l)
0	15
2	19
5	27.4
7	33.2
8	37.3
10,25	45.3
25.18	75.8

C. Measured ethanol and glucose concentrations

Ethanol and glucose concentrations measured during the fed-batch cultivations.

Table C.1 Fed -batch 8 ("blue" yeast)

time (h)	**Ethanol conc.** (mg/l)	**Glucose conc.** (mg/l)
1	7.7	51
2	87	72.5
3	21	73.4
4	26	104
5	23	95
6	36	113
7	37.8	101
10	24.2	87.7
22.5	14	40.6
23.5	25.6	38,4

Table C.2 Fed -batch 9 ("blue" yeast)

time (h) (h)	**Ethanol conc.** (mg/l)	**Glucose conc.** (mg/l)
1	12.8	98.9
2	15.8	102
3	16.4	61.3
4	20.5	80.7
5	320	83.3
6	20.2	84.2
7	20.2	84.2
11	21.3	60.9
23.17	24.1	36.3
24.5	31.4	44.5

Table C.3 Fed -batch 10 (“blue” yeast)

time (h)	**Ethanol conc.** (mg/l)	**Glucose conc.** (mg/l)
0	40	84.2
2	18.1	72.1
4	15.8	105
5	17.8	70.8
6	15.4	94.6
10.75	29	83.8
22	21.5	51.4
23	23.7	29.8
24.08	17	46.6

Table C.4 Fed-batch 11 (“blue” yeast)

Time (h)	**Ethanol conc.** (mg/l)	**Glucose conc.** (mg/l)
0	6.8	68.7
1	9.6	90.7
2	13.4	86.8
3	18.2	85.6
7	26.5	109
12	34.6	74.3
21	12.7	45.3
22	55.5	61.8

Table C.5 Fed-batch 17 (“blue” yeast)

Time (h)	**Ethanol conc.** (mg/l)	**Glucose conc.** (mg/l)
1	12.3	101
2	12.4	86.4
3	16	98
4	19.5	110
5	21.2	127
12	16.8	83.3
21.75	16	55.7
23.5	27	99.8

Table C.6 Fed-batch 15 (“red” yeast)

Time (h)	**Ethanol conc.** (mg/l)	**Glucose conc.** (mg/l)
1	4	80.8
2	8.3	83.8
3	13.7	81.1
4	9.3	74.3
5.5	65.8	49.7
6.5	12.2	76.9
7.5	17.5	61.3
9.5	31.2	50.1
13	26.3	39.3
24	17.2	42.3

Table C.7 Fed-batch 16 (“red” yeast)

Time (h)	**Ethanol conc.** (mg/l)	**Glucose conc.** (mg/l)
1	9	66.5
2	15.9	96.4
3	16.1	76.4
4	21.5	111
6	27.9	87.7
7	33.1	72.3
8	23.3	70
12.25	15.1	38
23.33	26.4	36.3
24.5	15.7	36.6

Table C.8 Fed-batch A (“blue” yeast), no pulse feeding.

Time (h)	**Ethanol conc.** (mg/l)	**Glucose conc.** (mg/l)
0	7	79.5
1	0	93.7
2	8.1	115
3	11.7	132
4	11.2	287
5	18.5	178
6	14	127
7	16.2	101
7.5	12.8	103
13	16	101
21.83	10.6	51.4
25	10.8	53.1

Table C.9 Fed-batch B (“blue” yeast), no pulse feeding.

Time (h)	**Ethanol conc.** (mg/l)	**Glucose conc.** (mg/l)
0	16.7	111
1	18.3	114
2	18.3	121
2.5	25.1	116
5	31.8	75.6
6	43.9	56.1
7	26	57.9
8	21.5	41.9
10.25	23.8	39.3
25.18	17.9	44

D. Calculated specific growth rates

Calculation of μ during the exponential feeding period. The length of the period differs between the fed batches.

Mass balances concerning cell mass:

$$\frac{dVX}{dt} = m \cdot X \cdot V$$

Eq D.1

$$\Rightarrow X \cdot \frac{dV}{dt} + V \cdot \frac{dX}{dt} = -m \cdot X \cdot V$$

$$\Rightarrow \frac{\frac{dV}{dt}}{V} + \frac{1}{X} \cdot \frac{dX}{dt} = m$$

$$\Rightarrow \frac{1}{X} \cdot dX = (-D + m) \cdot dt$$

$$\Rightarrow \ln(X_2) - \ln(X_1) = (-D + m)\Delta t$$

A $D_{average}$ is calculated in the area of concern and μ is derived from the slope of the curve ln(X) versus time (h). (μ-D) was calculated for the initial exponential phase.

Table D.1 Obtained results in the fed-batches. First for the "blue" yeast and then for the "red".

	μ (h^{-1})
Fed-batch 8	0.12
Fed-batch 9	0.12
Fed-batch 10	0.097
Fed-batch 11	0.12
Fed-batch 17	0.11
Fed-batch 18	0.12
Fed-batch A	0.11
Fed-batch 15	0.082
Fed-batch 16	0.11
Fed-batch B	0.13

E. Calculated yields during batch phases

$Y_{x/s}$ during the batch phases

Added cell mass through inocolum (g) = $(a \cdot Y_{XS})(b/100)$
a = glucose added in the inocolum (g) (always 3 g)
b = amount of inocolum added to the batch (ml)
Y_{xs} = Biomass / glucose yield (~ 0.2 g/g)

$$Y_{x/s}\ (g/g) = \frac{X_1 \times V - ((a \cdot Y_{xs})(b/100))}{m_g}$$

Eq E.1

V = batch volume (l)
X_1 = cell mass concentration at the end of the batch phase (g/l)
m_g = Amount of glucose added to the batch phase (g)

Table E.1 Y_{XS} during the batch phases. First for the "blue" yeast and then for the "red".

	$\mathbf{Y_{x/s}}$ (g/g)
Batch 8	0.33
Batch 9	0.31
Batch 10	0.28
Batch 11	0.30
Batch 17	0.30
Batch 18	0.29
Batch A	0.30
Batch 13	0.28
Batch 14*	0.28
Batch 15*	0.25
Batch 16*	0.26
Batch B	0.28

* 75 g of glucose were added during the batch phase. (Otherwise 59 g were added.)

The fed-batches 13, 14, 18 have not been referred to before, due to technical problems and computer errors. The batch phases though, were successful.

F. Calculated yields during fed-batch phases

Calculation of $Y_{x/s}$ for the fed-batch phases:

$$\text{Used glucose, } m_g \text{ (g)} = \left(1 - \frac{V_{2glucose-solution}}{V_{1glucose-solution}}\right) \cdot m_{0glucose} \qquad \text{Eq F.1}$$

$V_{1glucose\text{-}solution}$ = Initial volume of the feed solution. (ml)
$V_{2glucose\text{-}solution}$ = Final volume of the feed solution. (ml)
$m_{0glucose}$ = Amount of glucose added to the feed solution. (g)

Formed cell mass (g): $X_2 \cdot V_2 - X_1 \cdot V_1$ Eq F.2

X_1, V_1 = cell mass concentration (g/l) and medium volume (l) at the fed batch start,
X_2, V_2 = cell mass concentration (g/l) and medium volume (l) at the fed batch end)

Cell mass withdrawn through sampling has to be taken in consideration.

m_X (g) = (Formed cell mass) + (Cell mass withdrawn through sampling)

$Y_{x/s} = m_X / m_g$ Eq F.3

Table F.1 $Y_{x/s}$ -values for the fed-batches.

	$Y_{x/s}$ (g/g)
Fed-batch 8	0.44
Fed-batch 9	0.46
Fed-batch 10	0.44
Fed-batch 11	0.45
Fed-batch 17	0.48
Fed-batch 18	0.45
Fed-batch A	0.48
Fed-batch 15	0.50
Fed-batch 16	0.48
Fed-batch B	0.48

G. Calculation of K_La

K_La– calculations

H for oxygen at 298 K is $3.30 \cdot 10^7$ torr.
At a normal pressure (1 bar) the partial pressure of oxygen is 0.21 bars, which corresponds to solubility in water of $2.7 \cdot 10^{-4}$ M.

This corresponds to a 100% signal from the oxygen electrode.

$J = K_La(Co^* - Co)$ Eq G.1

J is the oxygen flux into the reactor.

Co^* is the maximal solubility in water, $2.7 \cdot 10^{-4}$ M (at 298 K).

We measure O, dissolved oxygen tension; the partial pressure corresponding to the dissolved oxygen concentration in the water. Thus O=100% corresponds to $Co=Co^*=2.7 \cdot 10^{-4}$ M

$\Rightarrow J = K_La(1 - (O/100)) \cdot 2.7 \cdot 10^{-4}$ mol/(l·h)

$J = F((y_{in} - y_{ut})/100)$ mol/(l·h)

V = Volume of the media.
F = Flow of gas through the reactor (mol/h)
y_{in} = Ratio of oxygen in the in gas (%)
y_{ut} = Ratio of oxygen in the out gas (%)

F must be converted from ml/min to mol/h.

$p \cdot V = n \cdot R \cdot T$ Eq G.2

$$F = \frac{p \cdot F^*}{R \cdot T} = \frac{1 \cdot \frac{F^* \cdot 60}{1000}}{0.082057 \cdot 303} \text{ mol/h}$$

$\Rightarrow F = F^* \cdot 0.00241$ mol / h

F^* is the gas flow (ml/min).

$$\Rightarrow J = \frac{F^* \cdot 0.00241 \cdot \frac{20.99 - y_{out}}{100}}{V} \text{ (mol/(l·h))}$$

Finally: $K_La = \frac{J}{\left(\frac{(100-O)}{100} \cdot 2.7E-4\right)}\ h^{-1}$ Eq G.3

H. Medium components

a. Vitamin solution

25 mg of d-biotin were dissolved in10 ml 0.1 M NaOH. Distilled water was added until the volume reached 400 ml. pH was adjusted to 6.5 (with 10% H_2SO_4). Then the following compounds were added:

p-Aminobenzoic acid (PABA)	100 mg
Nicotinic acid	500 mg
Calcium pantothenate	500 mg
Pyridoxine, HCl	500 mg
Thiamine, HCl	500 mg

pH was adjusted to 6.5 and then 12.5 g of m-Inositol were added. Again pH was adjusted to 6.5 and the volume was increased to 500 ml with distilled water. The solution was sterile filtered and poured into two autoclaved flasks. The solution was stored at 4° C.

b. Trace metal solution

EDTA	6.00 g
$CaCl_2 \cdot 2\ H_2O$	1.12 g
$ZnSO_4 \cdot 7\ H_2O$	1.80 g
$FeSO_4 \cdot 7\ H_2O$	1.20 g
H_3BO_3	0.40 g
$MnCl_2 \cdot 4\ H_2O$	0.244 g
$Na_2MoO_4 \cdot 2\ H_2O$	0.117 g
$CoCl_2 \cdot 6\ H_2O$	0.172 g
$CuSO_4 \cdot 5\ H_2O$	0.120 g
KI	0.040 g

The volume was adjusted to 2000 ml using distilled water. Afterwards pH was adjusted to 4.0 with 2M NaOH. The solution was autoclaved and then stored at 4º C.

I. LabVIEW program

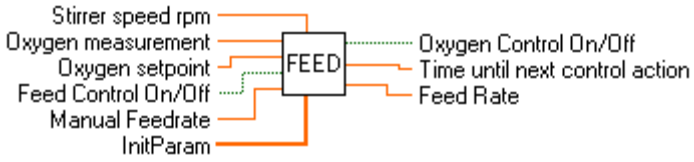
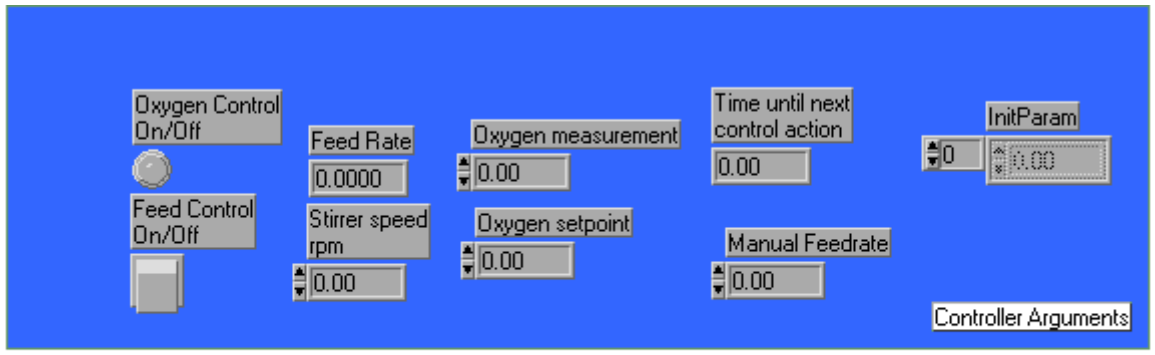


Figure I.1 Connection scheme



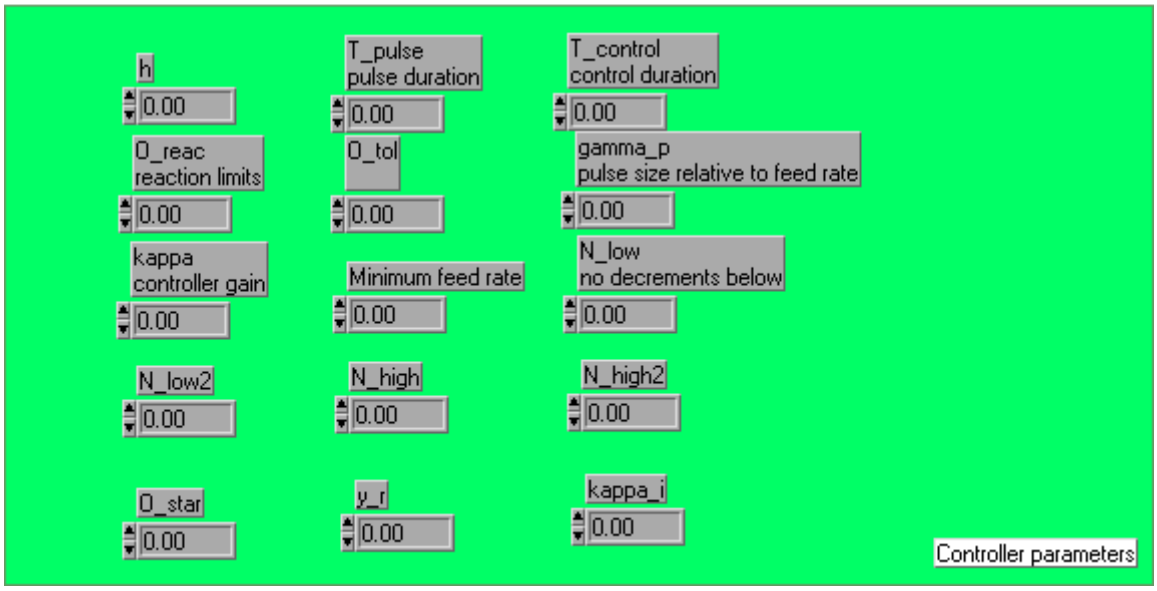
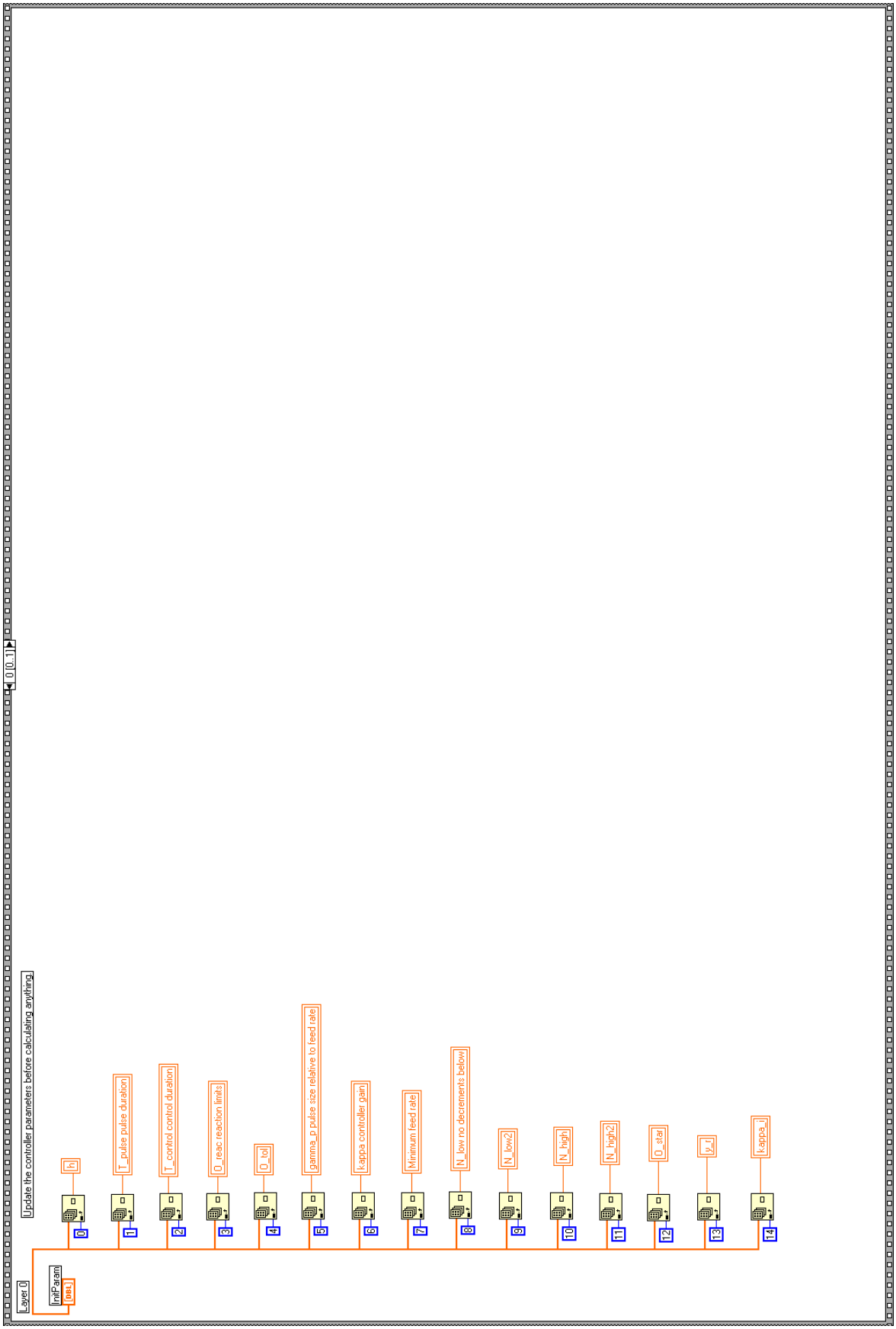
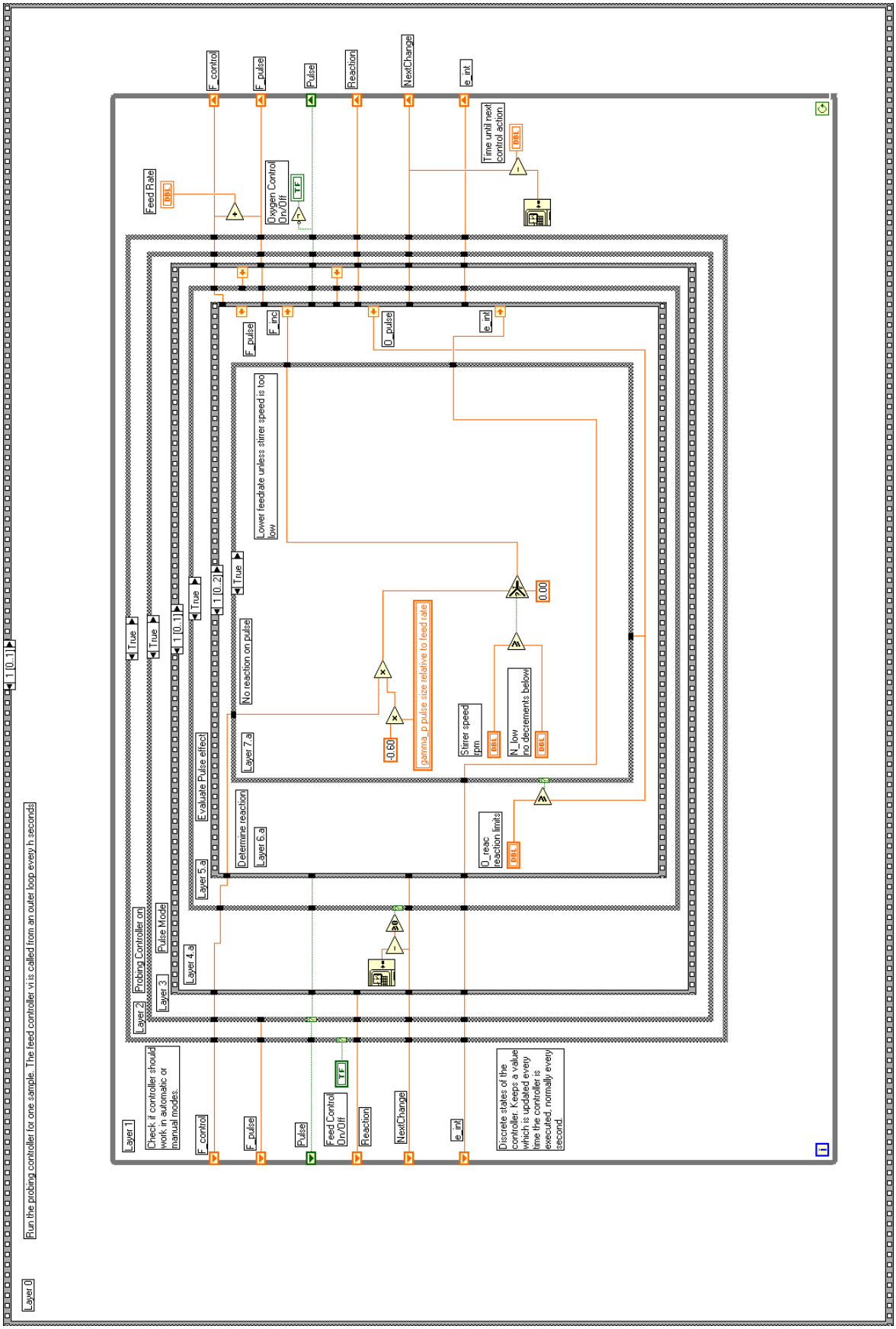
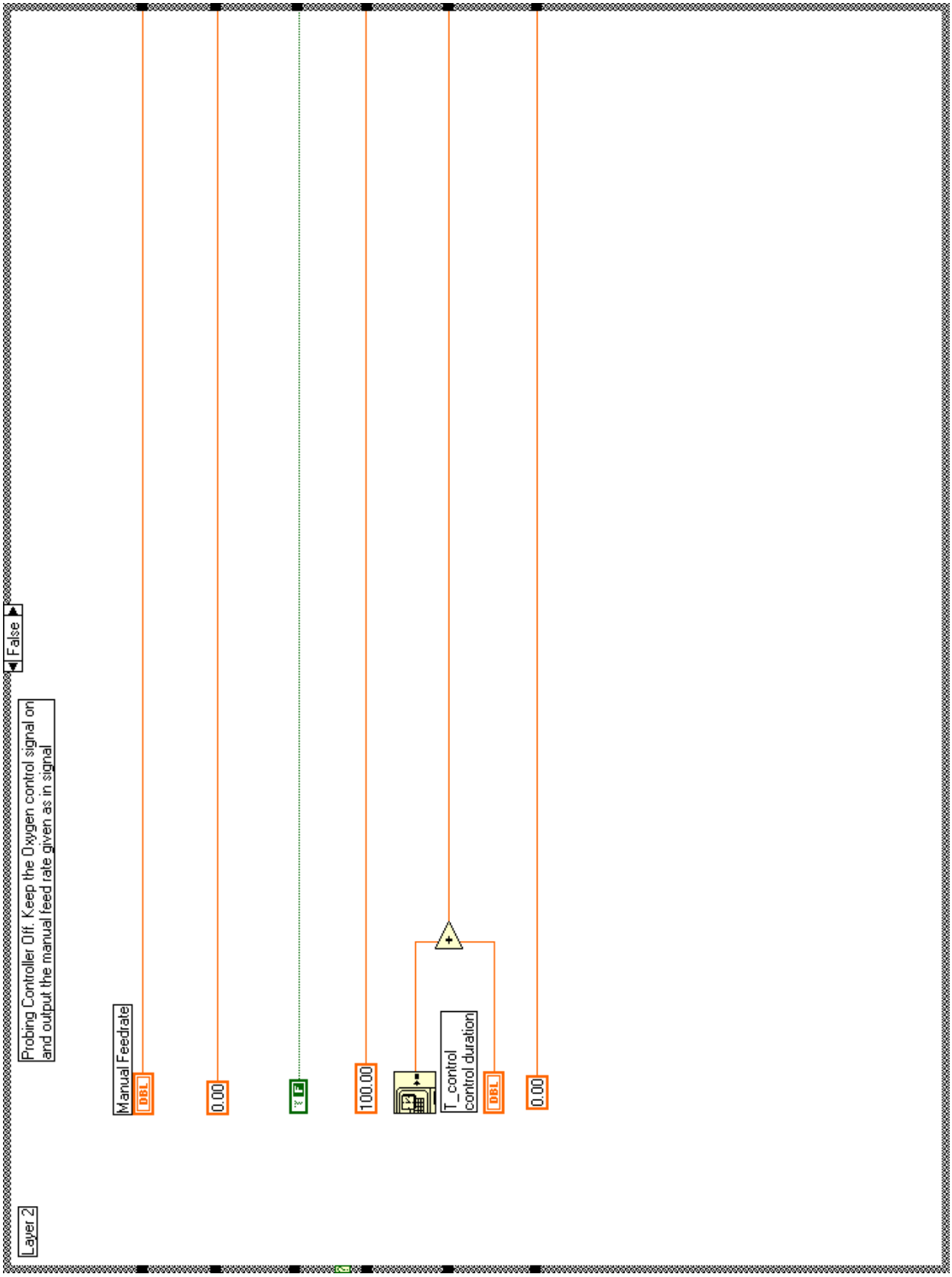


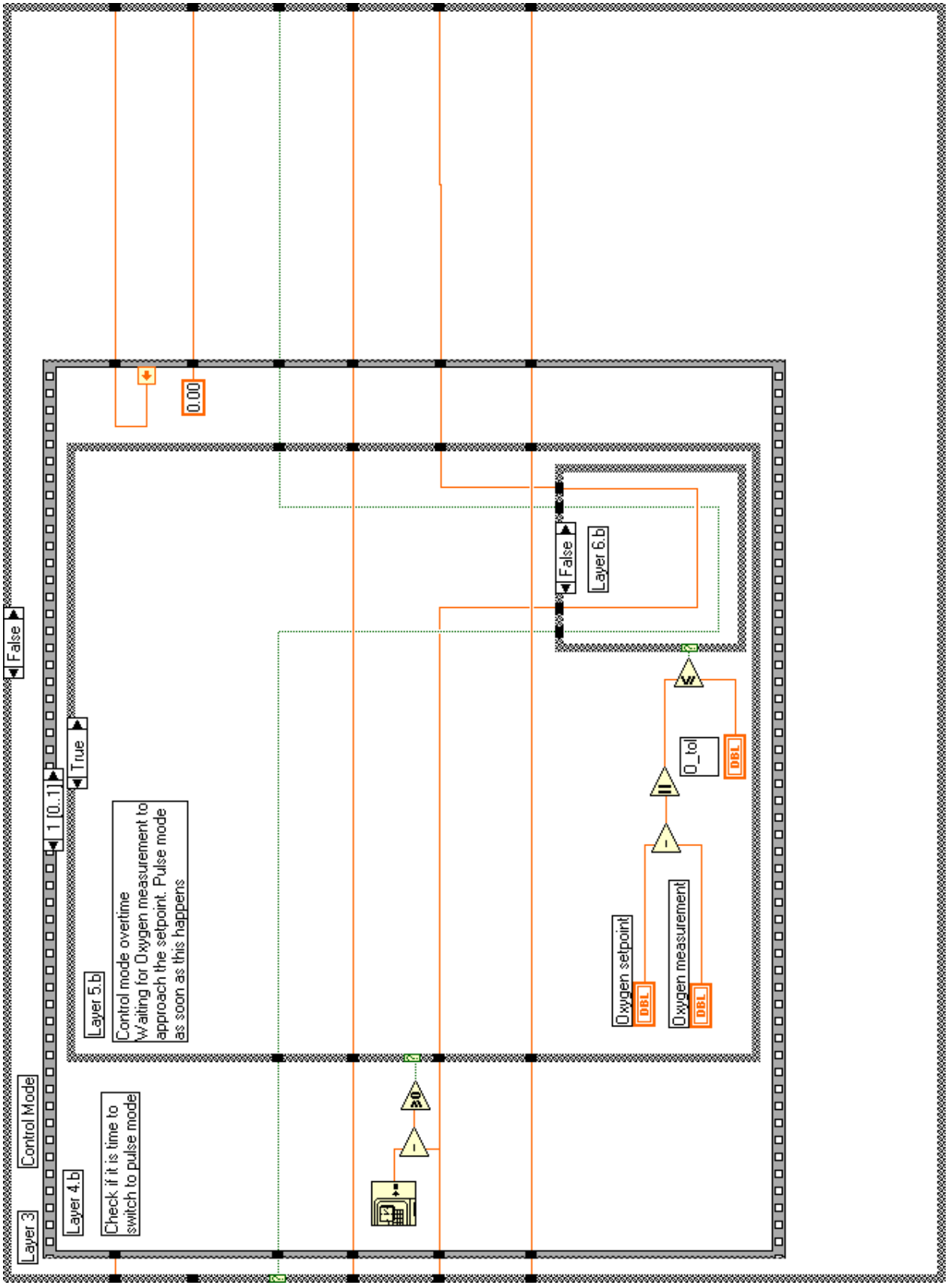
Table I-1 Descriptions of variables

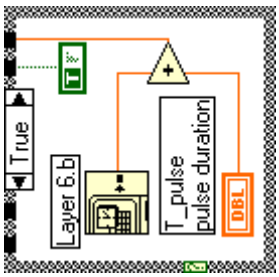
Type	Name	Explanation
TF	**Oxygen Control On/Off**	Signal to the stirrer PID controller to switch between hold and automatic mode.
DBL	**Manual Feedrate**	When not in automatic control mode, this feed rate will be used.
DBL	**Feed Rate**	Current feed rate. The output value from the controller.
TF	**Feed Control On/Off**	Switch between manual feed control and automatic feed control.
DBL	**Stirrer speed rpm**	Current stirrer speed in the reactor.
DBL	**Oxygen setpoint**	Setpoint level. Usually 30 %.
DBL	**Oxygen measurement**	Current measured dissolved oxygen level in the reactor.
DBL	**Time until next control action**	Time to next pulse / control period. If it is negative, the controller is waiting for Oxygen measurement to approach Oxygen setpoint.
DBL	**h**	Sampling period for the probing controller. Given in seconds.
DBL	**T_pulse pulse duration**	Pulse time in seconds.
DBL	**T_control control duration**	Control time in seconds.
DBL	**O_reac reaction limits**	The controller determines that an oxygen response to a pulse has happened if Oxygen measurement decreases below Oxygen setpoint-O_reac.
DBL	**O_tol**	Tolerance level. The controller doesn't issue a new pulse until Oxygen measurement is within Oxygen setpoint ± O_tol even if the control period is over.
DBL	**gamma_p pulse size relative to feed rate**	Pulse height is calculated as (1+gamma_p) multiplied with the current feed rate.
DBL	**kappa controller gain**	Proportional gain for calculating new feed rates from the oxygen responses during the pulses. Should be = 1 to guarantee stability.
DBL	**Minimum feed rate**	The controller never tries to decreases the feed rate below this value. It is not practically possible to use very small feed rates.
DBL	**N_low no decrements below**	No decrement in feed rate is allowed below this stirrer speed, to maintain a reasonable stirrer speed.
DBL	**N_low2**	Feed rate will be increased below this stirrer speed, to maintain a reasonable stirrer speed.
DBL	**N_high**	No increment in feed rate is allowed when stirrer speed is above N_high.
DBL	**N_high2**	The feed rate will automatically be decreased when stirrer speed is above N_high2.
DBL	**O_star**	The maximum amount of solvable oxygen in the medium. A percentage.
DBL	**y_r**	Oxygen response setpoint. The controller tries to keep responses around Oxygen setpoint – y_r.
DBL	**kappa_i**	Integral gain for calculating new feed rates.
[DBL]	**InitParam**	Controller parameters. Updated every time the controller vi is run.

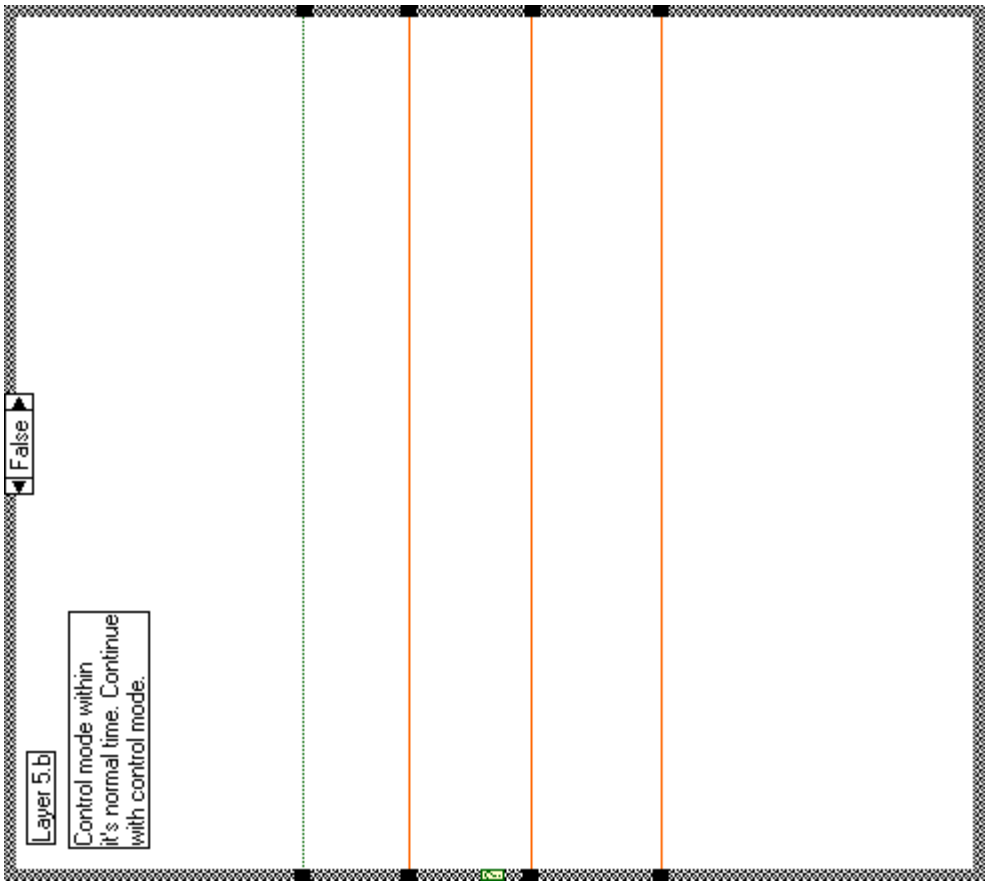

Update the controller parameters before calculating anything
Layer 0
inpParam
[0..1]
0
[0..1]
h
T_pulse pulse duration
T_control control duration
O_reac reaction limits
O_tol
gamma_p pulse size relative to feed rate
kappa controller gain
Minimum feed rate
N_low no decrements below
N_low2
N_high
N_high2
O_star
y_d
kappa_i
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

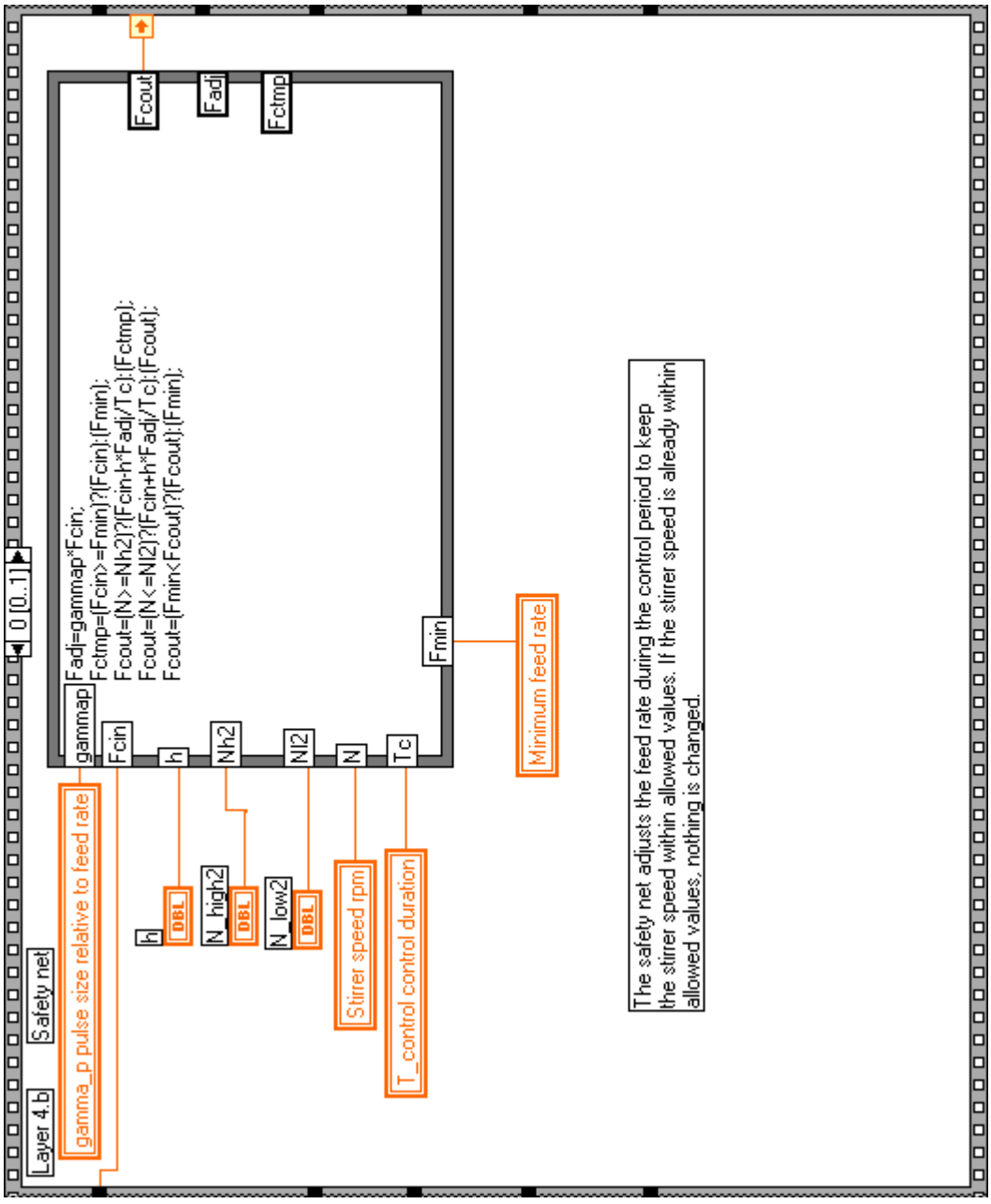

Layer 0
Run the probing controller for one sample. The feed controller vi is called from an outer loop every h seconds.
Layer 1
Check if controller should work in automatic or manual modes.
Layer 2
Probing Controller on
Pulse Mode
Layer 3
Layer 4.a
Layer 5.a
Evaluate Pulse effect
Determine reaction
Layer 6.a
Layer 7.a
No reaction on pulse
Lower feedrate unless stirer speed is too low
gamma_p pulse size relative to feed rate
-0.60
Stirrer speed rpm
N_low no decrements below
0_reac reaction limits
0.00
Discrete states of the controller. Keeps a value which is updated every time the controller is executed, normally every second.
F_control
F_pulse
Pulse
Feed Control On/Off
Reaction
NextChange
e_int
Feed Rate
Oxygen Control On/Off
Time until next control action
F_inc
0_pulse

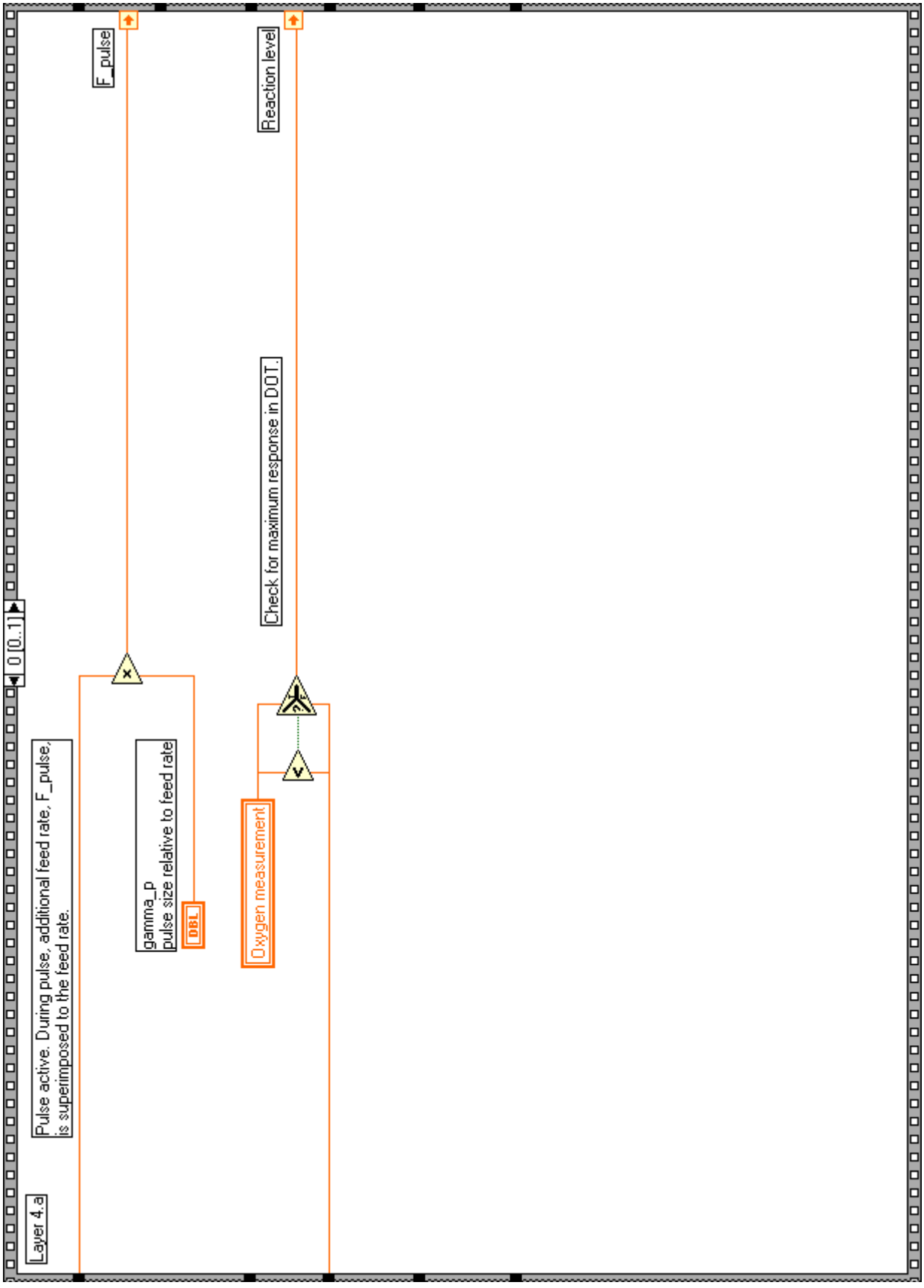

Layer 2
False
Probing Controller Off. Keep the Oxygen control signal on and output the manual feed rate given as in signal
Manual Feedrate
DBL
0.00
100.00
T_control control duration
DBL
0.00

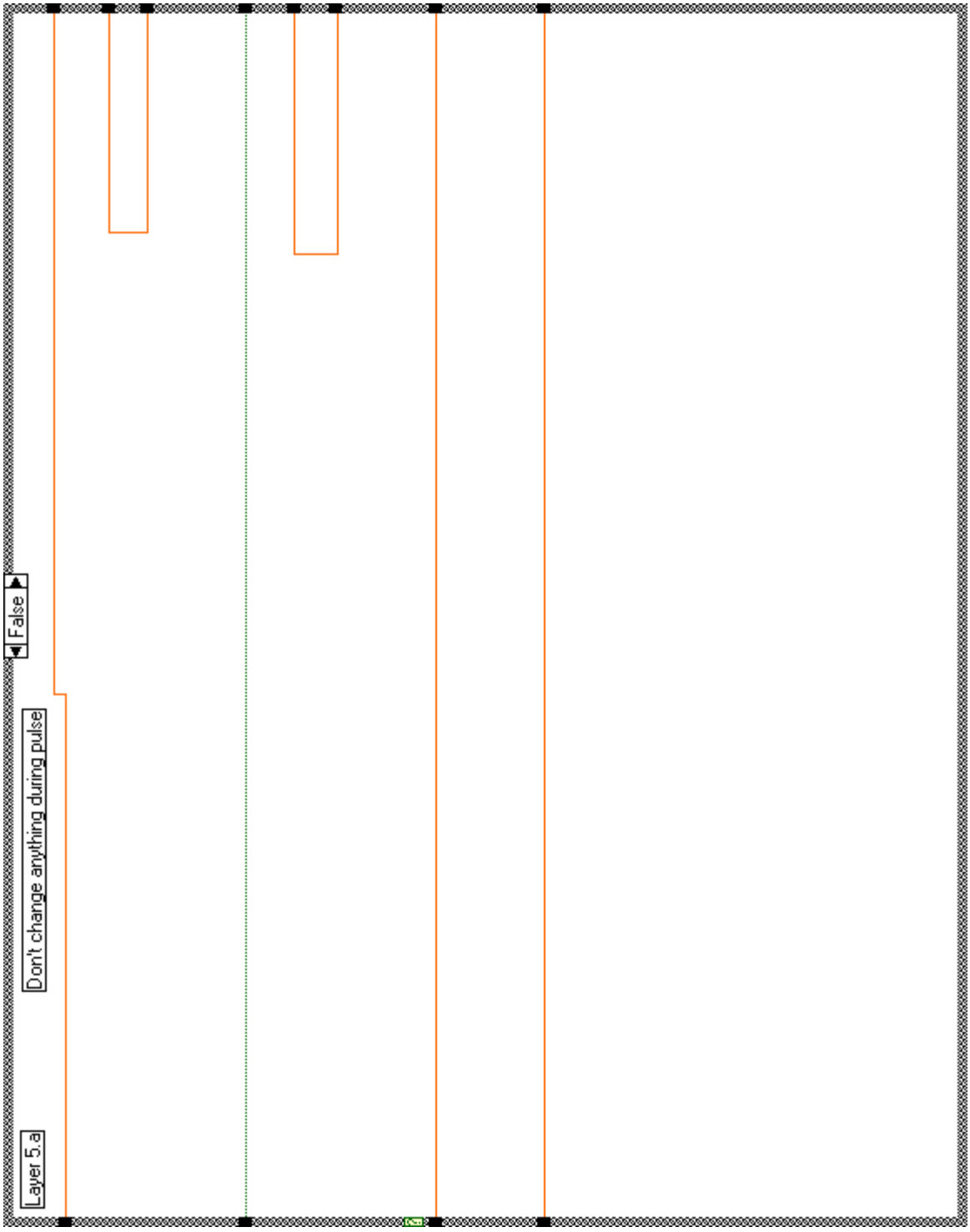

False
Control Mode
Layer 3
1 [0..1]
True
Layer 4.b
Check if it is time to switch to pulse mode
Layer 5.b
Control mode overtime
Waiting for Oxygen measurement to approach the setpoint. Pulse mode as soon as this happens
0.00
False
Layer 6.b
O_tol
DBL
Oxygen setpoint
Oxygen measurement

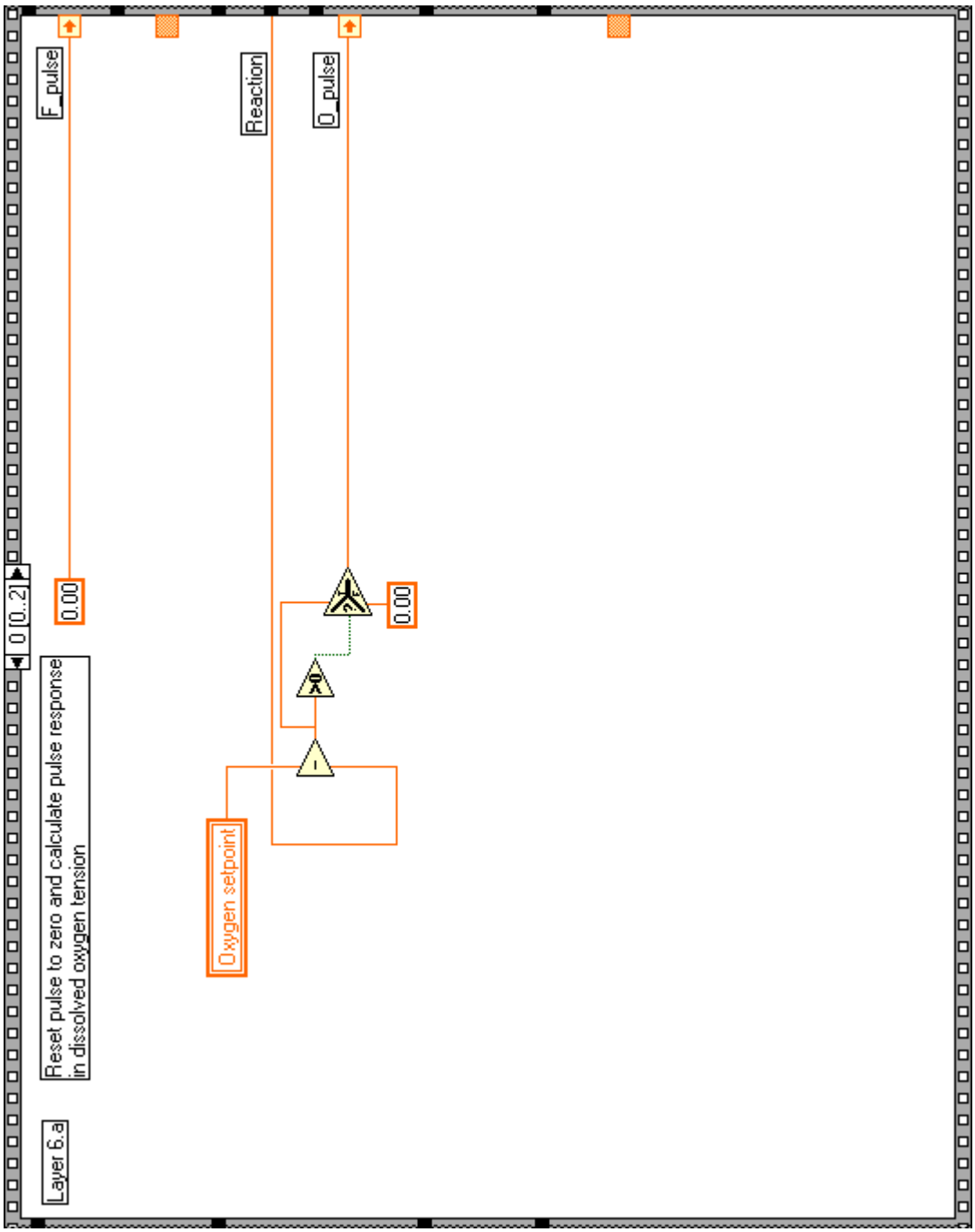

True
Layer 6.b
T_pulse
pulse duration
DBL

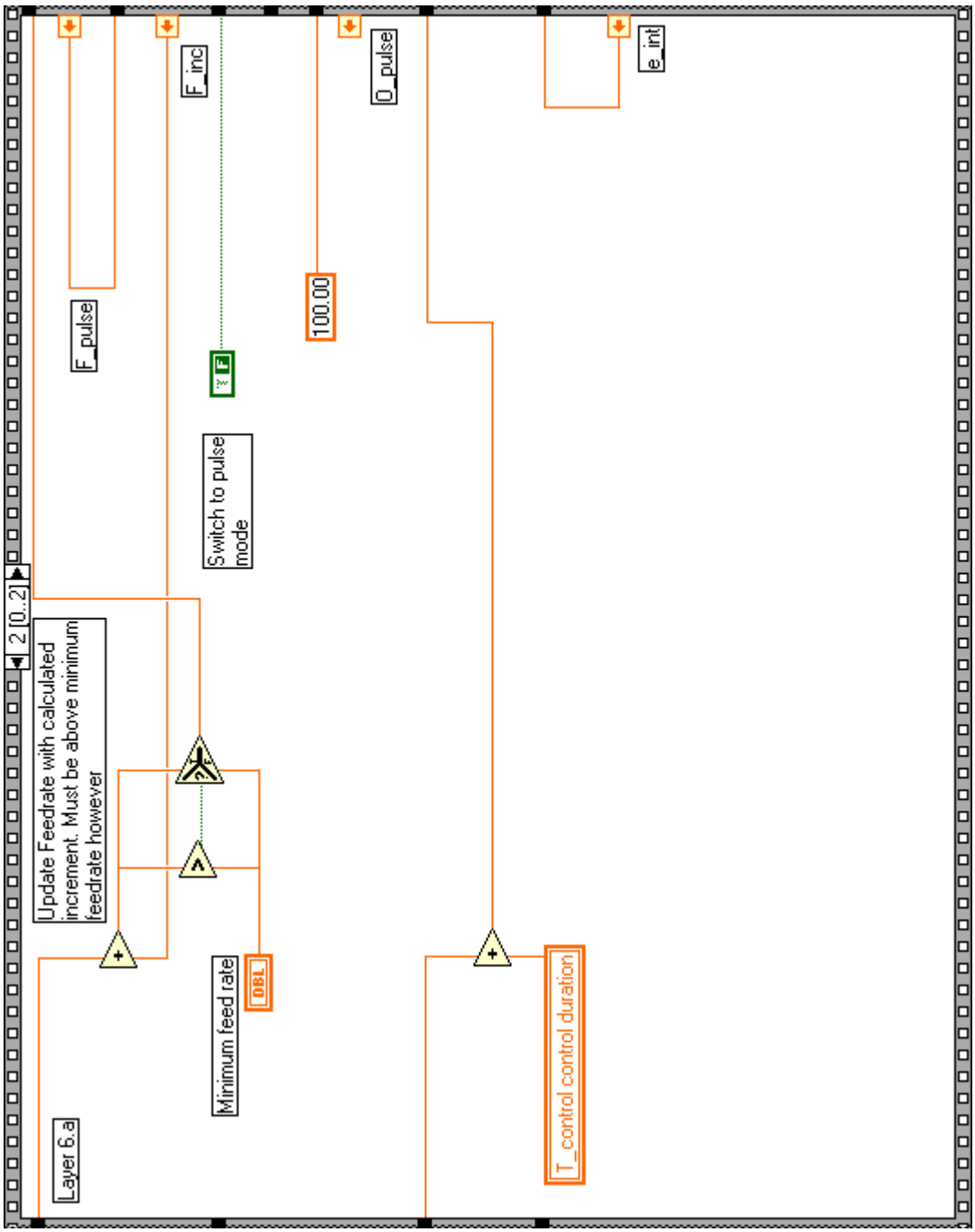

False
Layer 5.b
Control mode within
it's normal time. Continue
with control mode.

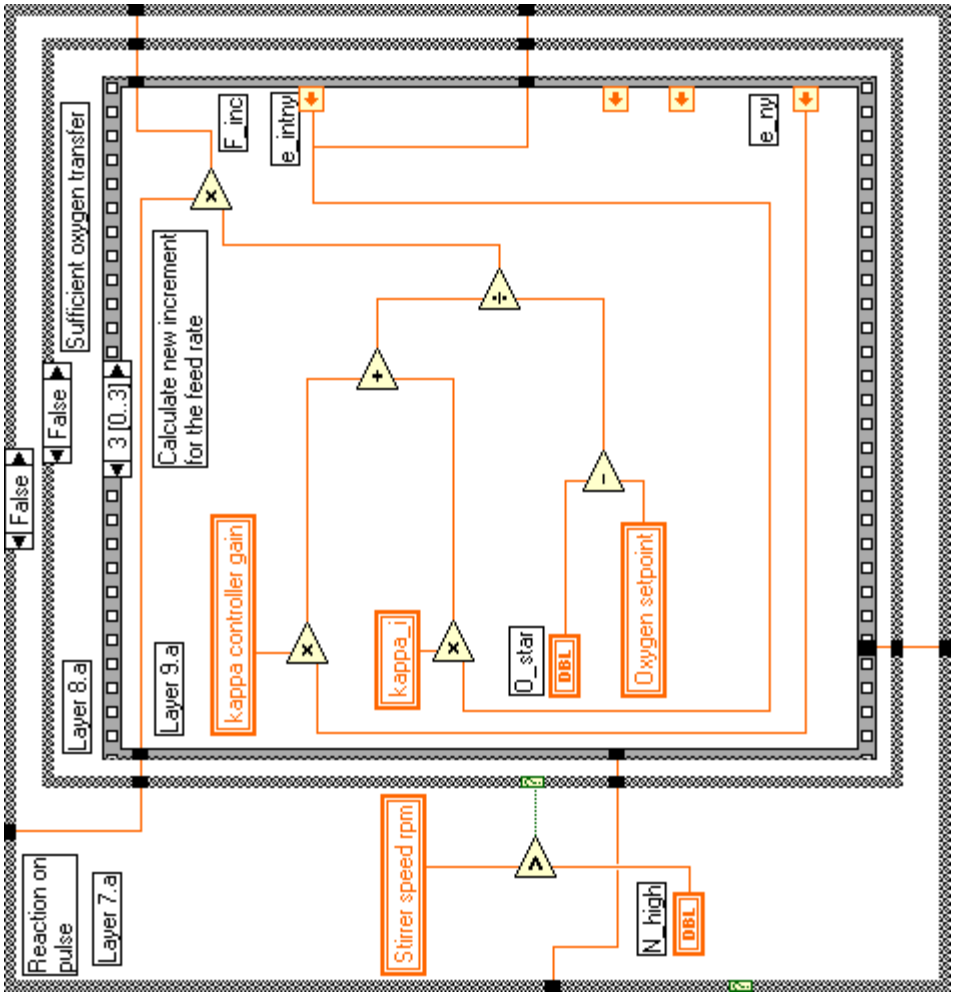

Layer 4.b
Safety net
gamma_p pulse size relative to feed rate
0 [0..1]
gammap
Fcin
h
Nh2
Nl2
N
Tc
Fadj=gammap*Fcin;
Fctmp=(Fcin>=Fmin)?(Fcin):(Fmin);
Fcout=(N>=Nh2)?(Fcin-h*Fadj/Tc):(Fctmp);
Fcout=(N<=Nl2)?(Fcin+h*Fadj/Tc):(Fcout);
Fcout=(Fmin<Fcout)?(Fcout):(Fmin);
Fcout
Fadj
Fctmp
Fmin
Minimum feed rate
h
N_high2
N_low2
DBL
Stirrer speed rpm
T_control control duration
The safety net adjusts the feed rate during the control period to keep the stirrer speed within allowed values. If the stirrer speed is already within allowed values, nothing is changed.

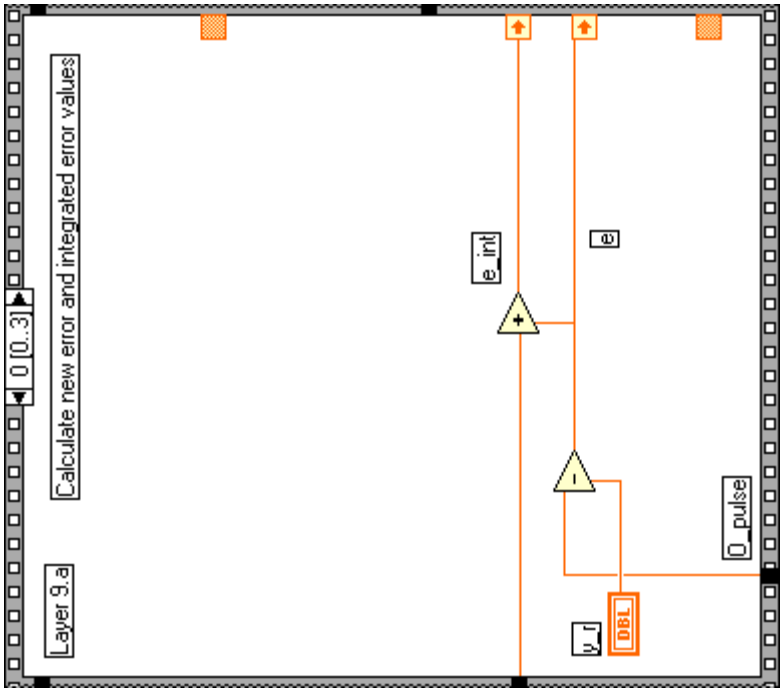

F_pulse
Reaction level
0 [0..1]
Layer 4.a
Pulse active. During pulse, additional feed rate, F_pulse, is superimposed to the feed rate.
gamma_p
pulse size relative to feed rate
DBL
Check for maximum response in DOT.
Oxygen measurement

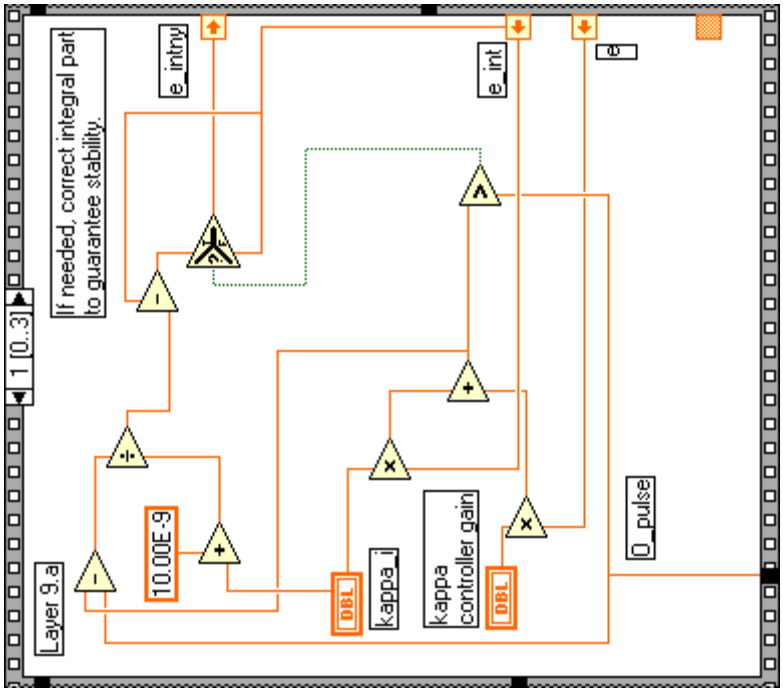

False
Don't change anything during pulse
Layer 5.a

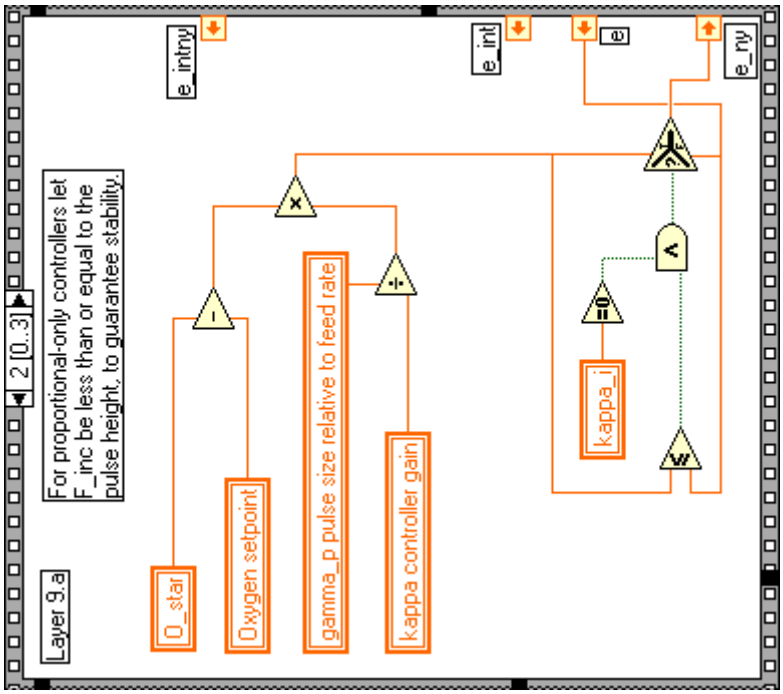

F_pulse
Reaction
O_pulse
0 [0..2]
0.00
0.00
Reset pulse to zero and calculate pulse response in dissolved oxygen tension
Layer 6.a
Oxygen setpoint

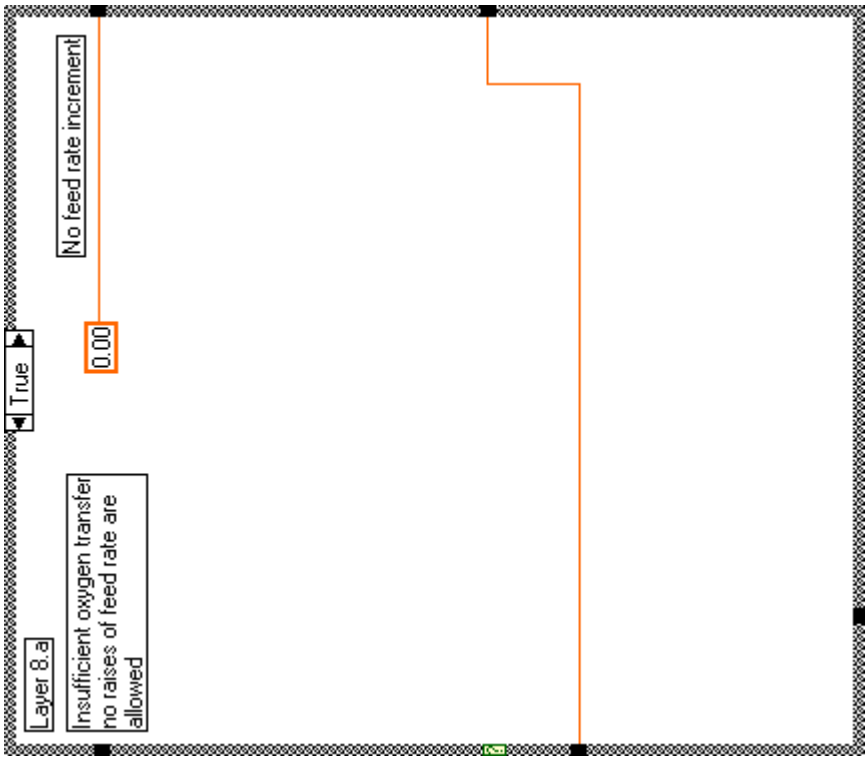

F_inc
O_pulse
e_int
F_pulse
100.00
Switch to pulse mode
2 [0..2]
Update Feedrate with calculated increment. Must be above minimum feedrate however
Minimum feed rate
DBL
Layer 6.a
T_control control duration


False
False
Reaction on pulse
Layer 7.a
Sufficient oxygen transfer
Layer 8.a
3 [0..3]
Calculate new increment for the feed rate
Layer 9.a
F_inc
e_intnyl
e_ny
kappa controller gain
kappa_i
O_star
DBL
Oxygen setpoint
Stirrer speed rpm
N_high
DBL


0 [0..3]
Calculate new error and integrated error values
Layer 9.a
e_int
e
O_pulse
y_i
DBL


1 [0..3]
If needed, correct integral part to guarantee stability.
Layer 9.a
e_intny
e_int
e
10.00E-9
DBL
kappa_i
kappa controller gain
DBL
O_pulse


2 [0..3]
For proportional-only controllers let F_inc be less than or equal to the pulse height, to guarantee stability.
Layer 9.a
e_intny
e_int
e
e_ny
O_star
Oxygen setpoint
gamma_p pulse size relative to feed rate
kappa controller gain
kappa_i


Layer 8.a
Insufficient oxygen transfer no raises of feed rate are allowed
True
0.00
No feed rate increment

J. Explanations of notations

ATP (Adenosine triphosphate) The main energy carrier molecule in a cell. The triphosphate group has a high chemical energy content, which can be released by hydrolysis of one phosphate-phosphate bond. ATP is used for many different purposes. For example: protein synthesis, transport of nutrient molecules across the cell membrane etc. ATP is formed in the respiratory chain, when a phosphate group is added to ADP, Adenosine diphosphate.

DOT (Dissolved oxygen tension, sometimes denoted DO). The partial pressure of oxygen corresponding to a certain dissolved oxygen concentration in water. The partial pressure of oxygen at a normal pressure is 0.21 bars, this corresponds to a dissolved oxygen conc. in the water of 2.7E-4 M. Often DOT is measure in %. 100% corresponds a dissolved oxygen conc. in the water phase of 2.7E-4 M.

$FADH_2$ (Flavine Adenosine dinucleotide) A coenzyme and electron carrier. (see NADH) When it is oxidized, the molecule releases one electron and two protons (hydrogen ions), and becomes NAD^+.

Glucose It is an organic molecule belonging to the group of carbohydrates. Glucose is a monosaccharide, meaning that it only consists of one sugar unit (sucrose consists of two, one glucose and one fructose unit). The molecule is hexagon shaped, with one carbon molecule in each corner. Chemical formula: $C_6H_{12}O_6$

K_La (The volumetric oxygen transfer coefficient). K_La actually consists of two parts: K_L and a. K_L is a mass transfer coefficient, determining the oxygen transfer through the thin laminar boundary layer surrounding an air bubble in water. The thickness of the layer is crucial for the k_L- value. A thin layer, for example at high stirrer speeds, give a relatively large K_L, while at low stirrer speeds the K_L is relatively small, due to a thicker boundary layer. a is the specific area (m^2/m^3) of the bubbles. When the stirrer speed is high the bubbles are broken down to smaller units, which makes a relatively big, compared to low stirrer speeds.

K_s (substrate saturation constant). The substrate concentration (in our case glucose), often in g/l, at which the specific glucose uptake rate, q_s, is half of the maximal specific glucose uptake rate, q_s^{max}. K_s is included in the Monod expression for the specific glucose uptake rate:

$$q_s = q_s^{max} \cdot \frac{S}{K_s + S}$$

Note that K_s has been used as a gain in the linearized transfer functions. In this context, it is not the same as the substrate saturation constant.

K_o (oxygen saturation constant), The oxygen concentration (g/l) in the media, at which q_o is half of q_o^{max}.
K_o has also been used as a gain in the linearized transfer functions.

NADH (Nicotinamide dinucleotide) This molecule is called a coenzyme, which means that even though it is not a protein, it is important because of its catalytic properties. The molecule is a so-called electron carrier. If an oxidation reaction takes place, the electron carriers absorb the electrons. They can be oxidized themselves (release their electrons), either in assisting reduction reactions (free electrons are needed in order for the reaction to proceed) or by being oxidized in the respiratory chain. When NADH is oxidized it releases one electron and one proton and becomes NAD^+.

μ (The specific growth rate). Could be called q_x but that is not the case. It describes the change in cell mass per amount of existing cell mass and hour (g/g·h). The growth of the cell mass can be written as $X = X_0 \cdot e^{\mu t}$. μ can be calculated from a plot, where ln(X) is plotted versus time. μ is then the slope of the curve. (X is cell mass concentration, g/l)

Respiratory chain A series of enzyme complexes. In yeast the respiratory chain is situated in the inner mitochondrion membrane. Electrons donated by NADH and $FADH_2$ are transported in a certain order between the enzyme complexes. The electrons are transported through a favourable potential difference and the potential energy released, can be used to pump hydrogen ions across the membrane, and thereby build up a potential difference over the membrane. This potential is the driving force, when new ATP molecules are formed by an enzyme in connection to the respiratory chain. The electrons are at last transferred to O_2, which is then reduced to water.

RQ (respiratory quotient) The RQ-value is the molar production of CO_2 divided by the molar consumption of O_2. At respiratory growth on glucose, the RQ value is close to 1.08 (Axelsson, 1989), which is easily explained by looking at the overall reaction (if the nitrogen flux were excluded the RQ-value would be 1.0):

$$C_6H_{12}O_6 + 2.33\ O_2 + 3.50\ NH_3 \rightarrow 3.5\ CH_{1.79}O_{0.57}N_{0.15} + 2.5\ CO_2 + 3.66\ H_2O$$

If the breakdown is totally fermentative the RQ-value can not be calculated because no oxygen is consumed. If only ethanol is consumed the RQ-value is only about 0.42 (if the nitrogen flux is included, otherwise 0.67)(Axelsson, 1989).

$$C_2H_6O + 1.61\ O_2 + 0.198\ NH_3 \rightarrow 1.32\ CH_{1.79}O_{0.57}N_{0.15} + 0.68\ CO_2 + 2.12\ H_2O$$

(The cell mass composition in the two reaction formulas was obtained from Sonnleitner and Käppeli, 1986.)

TCA-cycle (Tricarboxylic acid-cycle) A series of enzyme catalysed steps, where a six carbon compound is degraded to a four carbon one. Acetyl-coA enters the cycle (see figure 2-2) and directly joins oxaloacetate to form citrate. After a number of steps, where 2 CO_2 have been released, oxaloacetate remains. 3 NADH and 1 $FADH_2$-molecules are also formed.

Yield (denoted Y) A mass ratio between different components involved in the substrate breakdown and cell mass formation. $Y_{x/s}$ is the ratio between formed cell mass in grams and consumed glucose in grams. $Y_{o/s}$ is the ratio between grams of consumed oxygen, compared to grams of consumed substrate (in our case glucose). The Y-values do not remain the same, when the breakdown is changed from a respiratory to a fermentative status and vice versa. This explains why $Y_{x/s}^{ox} ? Y_{x/s}^{fer}$.