

## Co-Regulation Scheme Based on Decoupling of Interacting Loops for Fermentation Processes to Increase Bacteria Productivity

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Biomass production is strongly used in fermentation process like yogurts or beer manufacturing. Fermentation processes are most often operated while controlling pH, temperature, O<sub>2</sub> rate or oxydo-reduction (Redox) potential.

The production of biomass, as well as the stability of freeze-dried products, depends highly on the precision and robustness of the control scheme.

To optimize the production of biomass, it has been observed empirically that controlling both O<sub>2</sub> rate and Redox potential was the best strategy.

These two parameters are closely interacting with each other following Nernst law. It is then not reliable enough, from an industrial standpoint, to rely on the robustness of the control scheme to cope with this interaction.

The purpose of the presented work is first to characterize the interaction through the identification of the transfer function and then to define and implement a decoupling control scheme on a pilot bioreactor.

The results are significant with this method. The productivity has increased of 72% per operation and the biomass yield improves from 28%.

Nevertheless, some difficulties were met during experimentation due to sterilization of the material (probe).

### 1. Introduction

The industrial process we consider consists of micro-organisms growing inside a bioreactor of 0.8 US Gallon. Our innovation had to be applicable to lactic ferments or any other type of fermentation means that could be used for beer and wine production.

Regulating pH, temperature, O<sub>2</sub> rate will ensure sufficient growth of bacteria within the bioreactor (Ben-Naim, 2001). Empirical studies showed that the use of reducer has positive effect on growth. A second controller for the Redox potential has been added to the process. The selected reducer is hydrogen inside a gas mixture of N<sub>2</sub>H<sub>2</sub> (EP-1 856 241). The culture phase can last from 6 to 34 hours. During this time, the temperature, pH, O<sub>2</sub> rate and Redox are regulated. Setpoint for the O<sub>2</sub> rate and Redox potential are the same for all types of bacteria. Only the temperature and pH depend on the bacteria type. The biomass produced can be stored under liquid phase, by freezing, cryo-preservation or by freeze drying. The following picture shows the bioreactor:

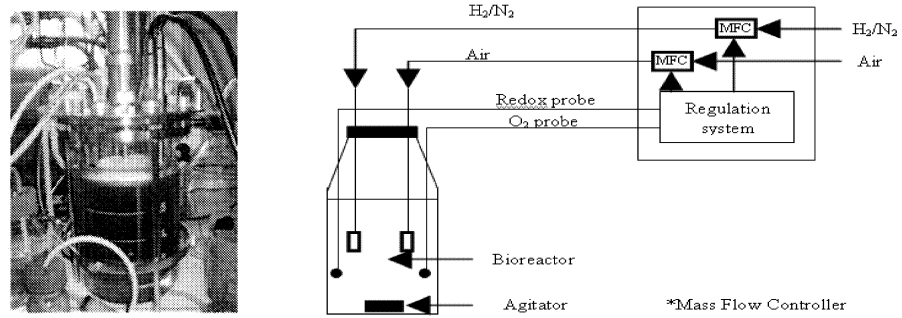


Figure 1 & 2: Picture of the pre-industrial set-up & figure of the fermentation process

## 2. Identification of Transfers Function

The following schema presents the manipulated variable (MV) and the controlled variables (CV) of the fermentation process:

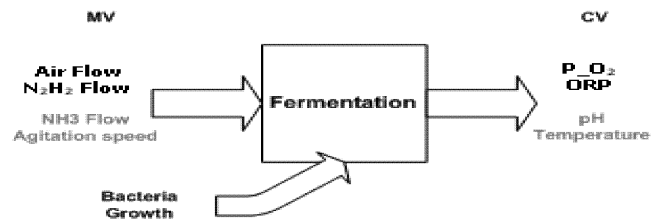


Figure 3: Overview of the variables

We take as input the oxidant gas flow (air) and reducer gas mixture flow ( $N_2H_2$ ) and as output the Redox potential and  $O_2$  rate.

The bacteria growth is seen as a perturbation since the controller action is highly depending on the biomass quantity and its corresponding activity ( $O_2$  consumption).

In grey, we show an example of “classic” variables controlled for any type of fermentation process and which is out of the scope of the presented work.

### 2.1 Effects of the air flow on Bacteria growing rate

To identify the effects of air injection on the process, a step test was performed.

The principle is the following: a continuous injection of air is done and all other regulators are switched off. We wait enough time to stabilize all controlled variables ( $P_{O_2}$  and ORP). The injection of air must be representative of the air flow reached during the bacteria growth. At this point, we change the air flow rate with a minimum value of 20 percent of the initial flow (it can be higher or lower flow rate). This sudden modification of the MV has an impact on the corresponding CV:  $O_2$  rate.

Below are the values stored during the step tests:

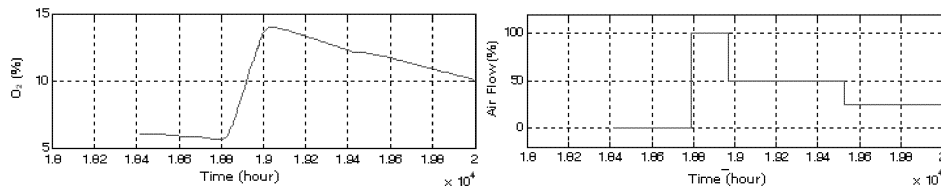


Figure 4: O<sub>2</sub> rate variation following step-test on air flow

The transfer function has been identified with Matlab using the “identification” toolbox. To improve the fit with the actual process response, the identification function has been tried with a first and a second order. Finally, the selected transfer function for air flow was chosen as a first order with constant delay, H<sub>1</sub>:

$$H_1(s) = \frac{K_1}{1 + T_{p1}s} e^{-T_{d1}s}$$

$$K1H = 0.13193 + -0.0073423$$

$$Tp1H = 84.364 + -6.9804$$

$$Td1H = 30$$

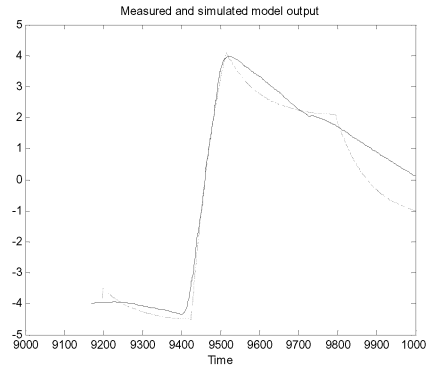


Figure 5: Transfer function H<sub>1</sub> (Air → P<sub>O<sub>2</sub></sub>)

### 2.2 Effects of the N<sub>2</sub>H<sub>2</sub> gas mixture on Bacteria growing rate

The same approach was performed on Redox potential.

The results obtained are:

$$G_1(s) = \frac{K_1}{s(1 + T_{p1}s)} ; K_{1G} = -0.022802 + -0.0016298 ; Tp1G = 470.28 + -69.627$$

### 2.3 Coupling effects

So far in this study, we have focused on the respective effects of one MV on the corresponding CV. With the step tests performed, we have observed an influence of each MV on all CVs of the process (especially on Redox potential and O<sub>2</sub> rate).

Another effect has been identified from the data: the injection of air has an impact on the two controlled variables. These coupling effect are modeled as follows:



Figure 6 & 7: Modelling Air effect on CVs & modelling N<sub>2</sub>H<sub>2</sub> effect on CVs

To be able to control the variables properly, the transfer function “H2” and “G2” have been characterized:

$$H_2(s) = \frac{K_2}{1 + T_{p2}s} e^{-T_{d2}s} \quad G_2(s) = \frac{K_2}{s(1 + T_{p2}s)}$$

$$K_{2H} = 0.8991 \pm 0.017165$$

$$K_{2G} = -0.0027402 \pm 0.00012985$$

$$T_{p2H} = 194.14 \pm 5.5399$$

$$T_{p2G} = 317.47 \pm 19.117$$

$$T_{d2H} = 18$$

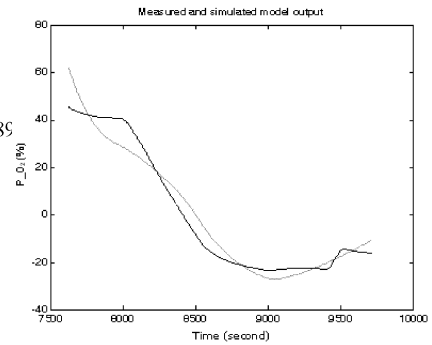


Figure 8: Transfer function H2 (Air → ORP) & Transfer function G2 (N<sub>2</sub>H<sub>2</sub> → P<sub>2</sub>O<sub>2</sub>)

The transfer function H2 and G2 are both first order types with positive integration term. This makes sense physically if we link this integration to the accumulation of product inside the bioreactor.

### 3. Control Scheme Decoupling both Variables

The global control scheme for manipulated and calculated variables linked to the O<sub>2</sub> rate and Redox is:

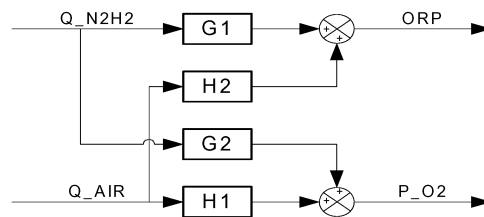


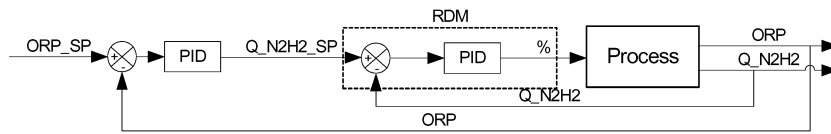
Figure 9: Decoupling control scheme

Further analysis has been done: it has been observed that the two transfer functions do not have the same format and gain.

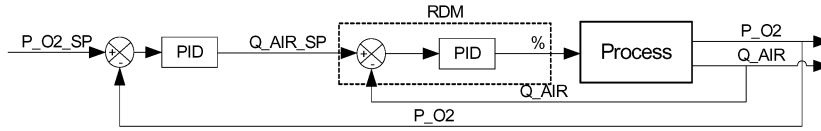
K<sub>1H</sub> is 48 times higher than the gain K<sub>2G</sub>. That's why the influence of N<sub>2</sub>H<sub>2</sub> is lower than the air on the O<sub>2</sub> rate. Besides, time constant for T<sub>p2G</sub> is 4 times higher than T<sub>p1H</sub>. Regarding the Redox potential, there is a factor 30 between the gain K<sub>2H</sub> the gain K<sub>1G</sub>. It is therefore possible to decouple the effects of the different transfer within the control scheme (W.-K. Chen, 1993).

It was then decided to use a simple architecture for the tests. Although the MIMO system (multi-input, multi-output) could be used, we preferred a two SISO (simple input, simple output) system based on Proportional Integrate Derivate (PID) controller.

The control structure implemented for the Redox potential was:



And for the  $O_2$  concentration:



The determination of the regulator parameters was done with RaPID software (from IPCOS) from step test data acquisition.

#### 4. Results and Optimization

The first test presents a complete growth of strain during 230 minutes.

Curves always show at least 3 phases:

Phase 1: Activation of the regulation system and stabilization of the controlled variables.

Phase 2: Growth implies an increase of the flowrate for the air and  $N_2H_2$ .

Phase 3: Growth is exponential. This is the phase where it is the most difficult to maintain constant the controlled variables.

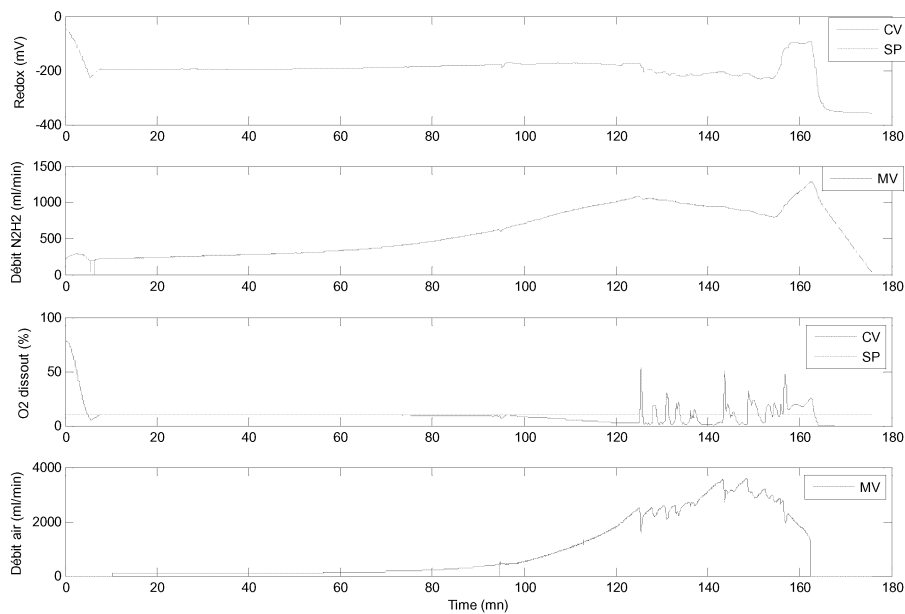


Figure 10: Tests results on strain B

## 5. Conclusions & Future Work

Compared to the initial control scheme, the co-regulation system has allowed reaching a gain up to 72% of productivity per operation. This productivity results by a gain up to 28% for the biomass yield.

On the considered bioreactor, in parallel to the work done on the regulation system, some issues were met due to the probe.

Such equipments need to be sterilized before using it in the bioreactor, in order to avoid growing of pollution bacteria. The issue is to find probe able to support the constraint of sterilization step.

The regulation during Phase 3 at the end could be improved by using logarithmic system control or conversion table applied on the output of the controller (to increase sensitivity of the regulation).

The interest of using O<sub>2</sub> instead of the air has not been studied neither yet on such processes (cf0).

We would like to mention that part of the work presented in this paper is protected within a patent.

## 6. Acknowledgment

We would like to thank Danisco R&D center at Dange St Romain (France) for their help in operating the process.

We also want to thank the Air Liquide R&D Bioressources team who contacted us in order to specify and implement the control solution for this process.

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