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PROJECT N" :
4522

PROJECT TITLE:

DEVELOPMENT OF NOVEL INTELLIGENT TECHNIQUES AND OPTIMAL ADAPTIVE CONTROL FOR FED-BATCH PENICILLIN FERMENTATION

START DATE:
1/4/92

DURATION:
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Keywords: Penicil I in fermentation, sampling devices, bio-sensors, mathematical modelling; control systems

i. SUMMARY

A sampling device, developed previously by SGI, was, after several experiments at the premises of the partners, improved to take samples from penicillin fermented broths continuously. However, despite the improvement of this device, due to several problems it had to be sometimes replaced by the ABC probe (ABC Biotechnologie/Bioverfahrenstechnik Gmbh, Germany), The samples from the sampling device or ABC probe could be analysed by two *enzyme* termistors, developed by ULUND, for glucose and penicillin in the broth.

A model was developed by UCL for the optimal control of penicillin based on adaptive control strategies which could include also software sensors for estimation of non-measurable fermentation variables. SGI developed the BIOAC control system which incorporated the software developed by UCL describing the penicillin fermentation model. The LT-control software of the Enzyme Thermistor (ET) (glucose and Penicillin) was connected to the MRU (Universal Regulation Module) penicillin and glucose modules allowing the BIOAC visualization and the ETs calibration. BIOAC uses these values for the control of the glucose feeding to the fermenter according to the software developed by UCL.

With the implementation of these harware and software elements into the bioreactors it was possible to control automatically the fermentation. The pilot validation of the complete system was done at CIPAN S.A. premises by running one fermentation with the control algorithm and another by the nominal (manual) control used at CIPAN, S. A. using an industrial strain of *Penicillium chrysogenum*.

It was demonstrated that the fermentation run with the control algorithm was superior in performance to the run with the nominal control of CIPAN, S A.. The figures below show the gain in productivity of the controlled fermentation in relation to the reference fermentation. The final titre of the controlled run was 31.2 g/l against 27.0 g/l oft he reference run giving a percentage of improvement of i 6%. The productivity (g PEN G/h) was 41.5 in the controlled run against 31.9 in the reference run giving a percentage of improvement of 30%. Finally, the product yield (g PEN G/g glucose) was 0.15 in the controlled run against 0.13 in the reference run giving a percentage of improvement of 15%.

2. OBJECTIVES OF THE PROJECT

The main goal of the project was the development of new monitoring techniques and advanced control methodologies to be applied on industrial fermentation processes ,

The case in study in the project was the *penicillin fed-batch fermentation.* It was expected to be able to produce new methodologies, with gains in maintenance. servicing and productivity on a real production system of penicillin validated at pilot scale and preindustrial level. Methodologies based on dynamical modelling would be used for improving and optimisation of overall system design, process automation and to enhance productivity and reliability in the penicillin manufacturing industry. Improved in-process condition monitoring capability by using new (bio)sensors giving required combination of cost, sensitivity and accuracy with achievement of' higher quality data would yield better description of the system and of its biological and technical performance Molecular analysis of catalyst could also result on improved selectivity. activity and lifetime for the production of penicillin by fermentation.

Specific objectives were, therefore:

1 *To improve the automatic sampling device*, so that it could operate with reliability *in* obtaining samples during at least 10 days, the time span of the fermentation. This would mean that new ceramic membranes and cleaning operation methods would be tried in order to avoid Fouling of the membranes in such a *viscous fermentation medium.*

2 *To connect new biosensors for glucose and penicillin* With *the* automatic sampling system to implement on-line analysis of these and consequently co extend the field of applications for this new device. Important objectives of this task were to have stable, reliable measurements and to decrease the time of sampling and *analysis to an* appropriate timing for the control action.

3. To *design "software sensors "* in order to compute estimates of biomass, active biomass and/or penicillin from available on-line measurements (gaseous inflows and outflows, glucose,...). The reconstructured fermentation variables are indeed difficult to be measured on-tine, but important for the process monitoring and control. Software sensors would be designed on the basis of ii physiological model of the penicillin fermentation obtained *from laboratory scale experiments*.

4. *To design* and *to implement adaptive optimal control strategies* especially adapted to secondary metabolites. The objective was to compute on-line on the *basis* of the physiological model, reactor feeding *profiles* which would self adapt to model uncertainties and which would allow to better keep the control of the process.

Implementation of the previous objectives using the "physiological" model description of the penicillin production a-reed at improving the control operation of the fermentation environment: additions, withdrawals, analysis. In this way, *a* typical time of 4 to 8 hours between off line sampling might be reduced to a few minutes with all the advantages d-rat it would have over the controllability of the fermentation and of the productivity itself Gains of productivity could therefore be expected either by increasing the "production" of the fermentation and possibly by increasing the product titer. In this way, final productivity gains might be increased by 5 to 10%.

3. MEANS TO ACHIEVE THE OBJECTIVES OF THE PROJECT

The means to achieve the objectives of the project for each partner are described below:

CIPAN S.A. (Partner 1)

Personnel:

- 1 PhD in Biochemical! Engineering Head of the Research and Development Department working for more than 20 years in the field of antibiotics production
- 1 PhD in Genetics heading the Microbiology Laboratory and Molecular Biology Laboratory working with CIPAN, S.A. for about 3 years
- 1 Agronomic Engineer specialised in Microbiology and Fermentation with 10 years experience heading the Pilot Plant Fermentation Installation
- 1 Agronomic Engineer specialised in Microbiology and Fermentation with more than 25 years experience heading the Fermentation Department

Facilities and equipment provided:

- Fermentation Pilot Plant installation comprising the following fermenters and pre-fermenters:
  1 x 9000 litres total volume fermenter equiped with automatic control system
  2 x 1050 + 2 x 540 litres total volume fermenters equipped with automatic control systems
  2 x 300 + 2 x 200 litres total volume pre-fermenters
  6 x 20 litres total volume pre-fermenters
- Microbiology Laboratory and Quality Control Laboratories equiped with all equipment necessary to manipulate cultures and to carry out in process control of the fermentations and analysis of CIPAN S.A. raw materials and finished products
- [n the scope of the proect, the following equipment was acquired with finding from the Comission:
  1 computer Schneider Euro SX 5 MHz
  1 Analyseur Glicose Microzym

SGI (Partner 2)

The main personnel from INCELTECH/SGI that was involved in the project was:

G. PIERRY	Management of the co-ordination between SGI and other partners from 4/1/1992 to 7/31/93

D.THEBLINE	Management of the co-ordination between SGI and other partners from 8/1/93 to the end of the project
J, N. RABAUD	Experimentation at SGI and assistance at INETI and collaboration with CIPAN, S.A.
P . PIERRON	Design of the sampling probe
J.F.DELAIGUE	Design of the 200 litres bioreactor provided to INETI
O.BERTEAU	Experimentation at SGI and assistance at INETI and collaboration with CIPAN,S.A.
D. PIRES	Assistance at INETI
F.LEME/ V. BARON	Experimentation at SGI with Glucose Analyser and trials on NADH determination by enzyme analyser
M. CAZAMAJOU	Design of the process control and data acquisition software
C. CASASNOVAS	4 months assistance at INETI

SGI has also used a sub-contractor to help in the development of the control system BIOAC.

Equipment and facilities used during the project:

- Mechanical design office including two computer development stations.
- Electronic design office including a computer development station.
- Computer development office including three software development stations.
- Mechanical workshop including milling machines, lathes. polishing machines, three stainless steel welding equipment and the assembly workshop
- Electrical/electronic workshop including mainly wiring station and settling stations
- Laboratory for microbiology and control including mainly analytical instrumentation such as HPLC, UV-VIS spectrophotometer, microscopes, balance and equipment for culture such as incubator, water bath, etc.
- Pilot hall allowing to work with fermenters of capacity up to 600 litres. This pilot hall is equipped with all the necessary utilities to run these fermenters (steam production, air production, cooling water, autoclave, oxygen, nitrogen, CO_2, etc.)

INETI (Partner 3}

Personnel

Dr.José Cardoso Duarte	PhD in Biochemical Engineering	
Dra. Ana Bossier	PhD in Biochemistry	Year 1 and 2
Pedro Noronha Pissarra	PhD Student	Year 1
António Pedro	Control Engineer	Year 1 and 2 (10%)
João Lourenço	Control Engineer	Year 3 (10%)
Cristina Barroso	Chemical Engineer	Year 1 and 2
Maria Leer-tor Rebelo	Biologist	
Maria Manuela Lageiro	BSc in Biochemical} Engineering	
João Mates de Sousa	BSc in Biochemical Engineering	

Facilities and equipment used during the project:

- Laboratory fermentation facilities for microbiological studies and scale-up to 100 litres fermenters.
- Analytical capabilities: HPLC, Gas Mass Spectrometer, NMR, GC, etc.
- Microbiological and Genetics Laboratories for support of the fermentation unit.

. Control Laboratory for developing and testing of so ftware and' hardware

- 200 L fermenter and accessories> including PC, 20 L fermenter, 2 Thermistor units, Glucose Analyser, automatic simpler, air compressor, steam generator and respective distribution lines.

UCL (Partner 4)

Scientific methodology:

The scientific methodology followed in this project was supported by the expertise in Mathematical System Theory and Control Science available in the Centre for Systems Engineering and Applied Mechanics (University of Louvain). Tools from various subfields of Control Science, such as System Identification, Optimal Control theory and Estimation theory, were used and combined in the study of software sensors and adaptive controllers for the penicillin fermentation process.

Computer facilities:

About thirty programs have been written for the project, using various appropriate languages such as MATLAB, FORTRAIN and C++. They were developed and run on a Sun workstation (Unix system) connected to the CESAME computer network. The facilities provided by the CESAME computer centre and the help from the operators of the centre were most usefull and well appreciated.

Personnel:

Six different persons were involved in the project. The main contributor was F. Jadot (PhD Student). He was helped by Dr. V. Van Breusegem (Senior scientist), Dr. BoYoung Hwang (Postdoc Fellow) and V. Chotteau (PhD student) at various stages of the project. Dr. J. Van [rope from the Department of Food and Microbial Technology of KUL was a technical advisor. The project was supervised by Prof. G. Bastin (Project Leader).

ULUND (Partner 5)

Personnel:

- 1 associate professor in Applied Biochemistry with almost 20 years experience *in* bioanalysis and biosensor technology.
- 1 PhD student in Applied BiochemistryBiotechnology

Equipment and facilities used during the project:

ULUND could offer the technical and scientific environment of an internationally well-known enzyme technology/biotechnology department belonging to one of Europe's largest chemistry institutions (The Chemical Centre). The specific equipment available was Enzymme Thermistors and their several accessories (computers, etc.)

4. MAIN RESULTS

4.1 Main results in the scope of objective 1

The starting point for this objective was the existing automatic sampling device of SGI-Sampler-F. With the aim of getting familiar with this device, connected to a 20 litres fermenter, an investigator from INETI spent two weeks at SGI premises. Then the sampler was transferred to INETI where it was tested with good results in relation to flow, but not in real fungi fermentations. Afterwards, the sampler was transferred to CIPAN, S.,4., where it was thoroughly tested in several different conditions in real penicillin fermentations. At CIPAN S.A. testing, some problems occurred concerning its handling and practical results, such as volume differences from sample to sample, broth contamination and significant differences between the analysis of the parameters of the penicillin fermentation measured in the samples taken directly from the fermenter and in samples taken by the SAMPLOR. Table I exemplifies these problems.

After several experiments were made, it was concluded that the reason for the differences in the analysis obtained in the broth taken from the fermenter and in the samples taken from the SAMPLOR was the dilution of samples. With a higher pre-sampling (from 0.6 to 3 ml) this problem was practically solved. according to SGI. However, the titration by SGI

probes revealed that some air bubbles could pass through the ceramic membrane which might cause damage to the Ets: This was apparently due to the membrane ageing and a new membrane would then be required for each fermentation. To be able to avoid any problems in using the SAMPLOR to take samples from the fermenter, the" ABC probe was tested at CIPAN, S.A. also in real penicillin fermentations. The results of such tests are shown in Table 2 and it could be concluded that the ABC probe was an alternative to the SAMPLOR as well as to the ceramic probes of SGI.

Table1 Comparative analysis obtained in fermentation 8/93 (Fermenter P 1, Standard volume 600 litres) with samples taken from the fermenter-and with SGI's sampling device.

Fermentation time (h)	Samples taken from the fermenter					Samplestaken with SGI's sampling device				
	Amonia Nitrogen (µg/ml)	Reducing sugars (%)	PAA (mg/ml)	Penicillin Chem. assay (U/ml)	Penicillin HPLC assay (U/ml)	Amonia Nitrogen (µg/ml)	Reducing sugars (%)	PAA (mg/ml)	Penicillin Chem. Assay (U/ml)	Penicillin HPLC assay (U/ml)
89	340	0.51	0.7P	10660	9743	180	0.39	0,68	90	Traces
98	250				-	250				-
113	240	0.63	0.60	15530	13838	145	0.49	0.63	3845	Traces
121	245	-	1.05		15060	180		0.80	-	1241
137	360	0.75	1.3s	17~7a	15779	225	0.57	1.41	4040	1338
161		0.84		20610		-	0.6.7	0.72	7050	
185	255	0.90	0.94	21790	20804	45	0.76		9595	10424
194	225	-			-	165			-	-
209	135	0.95		24190		45	0.82		42325	-
233	270	1 02		25745	-	150	0.82		10440	-

Table2 Comparison between standard and ABC probe sampling for Fermentation 15/94.

TIME	AMMMONIA NITROGEN (µg/ml)		GLUCOSE (g/l)		PAA (mg/ml)		PENICILLIN CHEMIC L ASSAY (U/ml)		PENICILLIN H C (U/ml)	
(h)	Standard	ABC Probe	Standard	ABC Probe	Standard	ABC Probe	Standard	ABC Probe	Standard	ABC Probe
07			1.79	1.81						
21	980	965			0.49	0.48				
25	975	935	0.43	0 44						
45	360	370			0.76	0.78	2745	2480	2150	2145
49	260	265	0.4 I	0.42						
69	165	160			0.74	0.74	6965	7210	7150	7200
73	210	205	0.29	0.22						
93	130	135			0.76	0.75	12200	12155	10734	10502
97	155	135	0.29							
117	295	235			0.83	0.85	15835	15555	14950	14920
121	235	220	0.43	0.44						
141	225	205			0.80	0.s0	18305	19005	19600	19130
145	245	205	0.43	0.42						
165	225	200			0.87	0.96	21990	21430		
169	235	200	0.49	0 50						
189	310	280			0.72	0.72	24775	23380	21300	20650
193	390	345	0.53	055						
213	705	660			0.92	0.95	24030	22910	20485	20385

4.2 Main results **in the scoped objective** 2

Upon receptions of the Enzyme Thermistor on 16 February 1993 at INETI, the thermistor prototype with an enzyme packaging for penicillin and glucose analysis in a dual-channel instrument containing two immobilised enzyme columns was assembled. The first experiments for penicillin and glucose determination with the ET with a penicillinase immobilised column and glucose-oxidase/catalase immobilised column were carried out in samples withdrawn from a chemostat culture. Stabilisation, calibration and tests of the device were done and a good linearity between the height of the peaks and the concentration oft he standards was observed,

The designed biosensor system uses a LT-CONTROL software for automatic control of a Rheodyne valve (model 5020) through the actuators (Universal ANACHEN ACP 3010) and it also does the penicillin and glucose concentration data acquisition. This software *is* connected *to the* MRU *penicillin and glucose* modules, allowing the BIOAC visualization and ET calibration of penicillin and glucose concentrations. BIOAC will use these values for the control of the glucose feeding to the fermenter. The LT-CONTROL programme was developed by Thomas Carlsson from the University of Lund during his staying at INETI. This programme allows to choose the sampling period and ET penicillin/glucose calibration, Different calibration methods were tested and the use of internal standard was the one preferred, The penicillin biosensor in the split flow injection operating mode was also tested,

Due to technical difficulties with the SAMPLOR, referred above, its connection with the ET was not attempted. However, the implementation of the system was done with the ABC sampling probe, gently supplied by the ULUND group, This probe. with a polypropylene membrane filter had already been tested on penicillin fermentations and was also tested on such fermentation by CIPAN, S. A. as reported above.

A software package was developed to use with the on-line analysis equipment. The software and hardware interfaces were tested in order to be implemented in the 200 litres pilot plant fermenter. With the purpose of experimental data *storage* and visualization, a data acquisition program was developed. The automatic, on-fine, sampling acquisition system was *implemented and tested* at INETI for on-line measure of penicillin and glucose concentration by biosensor systems. The on-**line sampling** system could use two types of probes with continuous pumping: the SGI ceramic probe (O. 2 μm) or the' symmetric micro-filtration membrane made of polypropylene, ABC probe. The SGI SAMPLOR was not used as refereed above. The sampling acquisition system was, therefore, implemented at INETI for on-line" measurement of penicillin and glucose concentration by biosensor systems.

4.3 **Main results in the scope of objectives** 3 **and** 4

These objectives would imply the optimization of the CIPAN, S.A. penicillin fermentation process. The CIPAN, S.A. process takes place in an aerated stirred tank reactor as shown in Figure I.

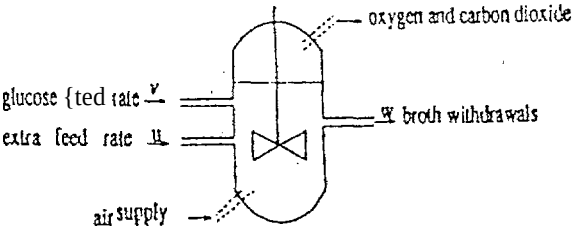

Figure 1 Process diagram

Glucose is fed to the reactor with a volumetric flow rate v. The other influent species are fed to the reactor separately with an overall volumetric flow rate u. Broth withdrawals are also operated: all the species are withdrawn at the same volumetric flow rate w. From experimental data, an underlying reaction network of the CIPAN, S.A. penicillin fermentation can be identified as follows:

$$S \rightarrow X \quad (R1) \qquad S \xrightarrow{X} P \quad (R2)$$

where S, X, P represent glucose, biomass (*Penicillium chrysogenum*) and penicillin-G, respectively. The first reaction (R1) corresponds to the *trophophase*, that is the phase of rapid biomass growth. The second reaction (R2) corresponds to the

idiophase, that is the subsequent phase of penicillin biosynthesis. A mathematical model of the CIPAN, S. A. process can then be derived from mass balance considerations:

$$\dot{C}_s = -\sigma C_x - \frac{u+v}{V} C_s + \frac{v}{V} C_s^{in} \quad (1)$$

$$\dot{C}_x = \mu C_x - \frac{u+v}{V} C_x \quad (2)$$

$$\dot{C}_p = \pi C_x - \frac{u+v}{V} C_p \quad (3)$$

$$\dot{V} = u + v - w. \quad (4)$$

where V denotes the broth volume, $\dot{C}_s, \dot{C}_x, \dot{C}_p$ denote the concentrations of the species S, X, P respectively, w, v, u denote the rate of broth withdrawals, the glucose feed rate and the extra feed rate respectively, C_s^{in} denotes the glucose influent concentration, μ, π denote the specific rates of growth and production respectively, $\sigma = \mu/Y_{x/s} + \pi/Y_{p/s}$ denotes the glucose consumption rate with $Y_{x/s}, Y_{p/s}$ denoting the yield coefficients. There is an evidence from the literature to select the model with Michaelis-Menten kinetics to account for the glucose activation of the biomass growth and Haldane kinetics to account for the glucose inhibition of the penicillin production, i.e.

$$\mu(C_s) = \mu_m \frac{C_s}{K_s + C_s} \qquad \pi(C_s) = \pi_m \frac{C_s}{K_p + C_s + C_s^2/K_i}$$

with $\mu_m, K_s, \pi_m, K_p, K_i$ denoting the kinetic coefficients. The parameter values of the model (l)-(4) resulting from an identification procedure, which allows a decoupling between the estimation of the yield coefficients and the calibration of the kinetic coefficients, are presented in Table 3. The model (1)-(4) with the parameter values of Table 3 is effectively able to describe the behaviour of the CIPAN, S.A. process as shown in Fig.2. It will be used 3s the *nominal model* of the process in the sequel

Table 3 Parameter values

$Y_{x/s} = 0{,}99$ (g/g)	$Y_{p/s} = 0.16$ (g/g)	
$\mu_m = 0{,}13$ (h^{-1})	$K_s = 1$ (g/l)	
$\pi_m = 0.005.(11^{-1})$	$K_p = 0.001$ (g/l)	$K_i = 10$ (g/l)

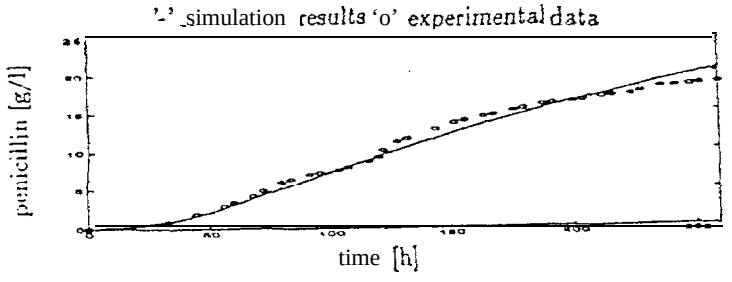

Figure 2 Fermentation 'J S-90

For the optimisation of the process, two performance criteria are considered:

- the average productivity J_p which is the ratio between the harvested amount of penicillin and the duration of the fermentation.
- the average yield J_y which is the ratio between the harvested amount of penicillin and the added amount of glucose.

In order to maximize J_p and J_y, the following optimal control sequence for the glucose feeding, in three phases, is recommended:

- Lag phase ($0 \le t \le t_o$): the process is operated in batch mode (u = v = w = 0) according to the CIPAN, S.A. operating mode.
- Growth phase ($t_o \le t \le t_s$): the process is operated in fed-batch mode with a constant glucose feed rate $v = v_m$. The goal is to accumulate the biomass as fast as possible. The amount of glucose devoted to the growth phase is denoted The goal is to accumulate the biomass as fast as possible. The amount of glucose devoted to the growth phase is denoted $S^* = C_s^{in} v_m (t_s - t_o)$.
- Production phase ($t_s \le t \le t_f$): the process is operated in fed-batch mode with a glucose feed rate v controlled to maintain the glucose concentration C_s at a fixed set point C_s^*. The goal is to increase the production rate by selecting a small value for C_s^*.

On the other hand, it is suggested to operate *continuous* broth withdrawals in order to remain at the maximum reactor capacity. The glucose feed rate v_m during the growth phase is constrained in order to avoid oxygen limitation effects. The criteria J_p and J_y are represented with the respect to the parameters S^* and C_s^* in Figures 3 and 4.

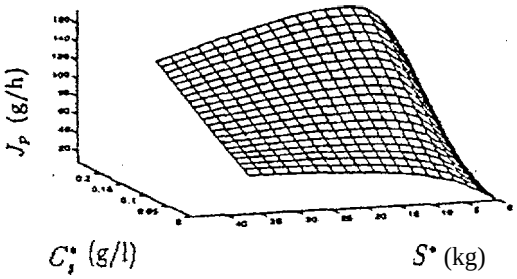

Figure 3 Productivity

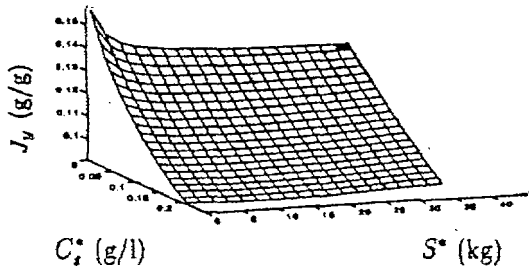

Figure 4 Average yield

The following conclusions can be drawn from these figures:

1. a conflict between yield and productivity is clearly apparent: for a given S^*, J_p is an increasing function of C_s^* while J_y is a decreasing function of C_s"
2. the dependence of J_p and J_y on S^* is more intrincate: in particular, it appears that there is an optimal value of S^* that maximizes J_p while J_y is rather insensitive to variations of S^*.

As a trade off between yield and productivity, UCL then proposed to *maximise* the productivity while keeping the yield constant. From a simulation experiment, it was shown that an increase of the productivity J_p by 10°/0 can be expected when the yield is fixed at J_y = O. 13. For the monitoring of the process, the following software sensor was developed for the on-line estimation of the biomass concentration (which is not measured on-line) and the estimation of the specific growth and production rates (which are not measurable)

$$\dot{\hat{C}}_x = \hat{\mu} C_x^{calc} - \frac{u+v}{V} C_x^{calc} + \frac{2}{T}(C_x^{calc} - \hat{C}_x)$$

$$\dot{\hat{C}}_p = \hat{\pi} C_x^{calc} - \frac{u+v}{V} C_p^{m} + \frac{2}{T}(C_p - \hat{C}_p)$$

$$\dot{\hat{\mu}} = \frac{1}{T^2} \frac{C_x^{calc} - \hat{C}_x}{C_x^{calc}}$$

$$\dot{\hat{\pi}} = \frac{1}{T^2} \frac{C_p - \hat{C}_p}{C_x^{calc}}$$

$$\dot{\hat{Z}} = -\frac{u+v}{V} \hat{Z} + \frac{v}{V} C_s^{in}$$

$$C_x^{calc} = Y_{x/s}(\hat{Z} - C_s - \frac{1}{Y_{p/s}} C_p)$$

where $\dot{\hat{C}}_x$, $\dot{\hat{C}}_p$ denote the on-line estimates of the biomass and penicillin concentrations respectively, $\hat{\mu}, \hat{\pi}$ denote the on-line estimates of the specific growth and production rates respectively, $\hat{Z}$ denotes the on-line estimate of the auxiliary variable $\hat{Z} = C_s + C_x/Y_{x/s} + C_p/Y_{p/s}$ denotes an alternative ort line estimate of the biomass concentration, C_s^m, C_p^m denote the on-line measurements of the glucose and penicillin concentrations respectively, while T can be considered as the estimation time constant. The tuning of T results from a trade off as shown in Figure 5 between the speed of convergence represented by the mean square observation error e_{obs} with respect to biomass dry weight data and the sensitivity to noise represented by the relative measurement noise η on *glucose* and penicillin data.

The following conclusions can be drawn from this figure:

1. as expected, e_{obs} is an increasing function of η for a given T.

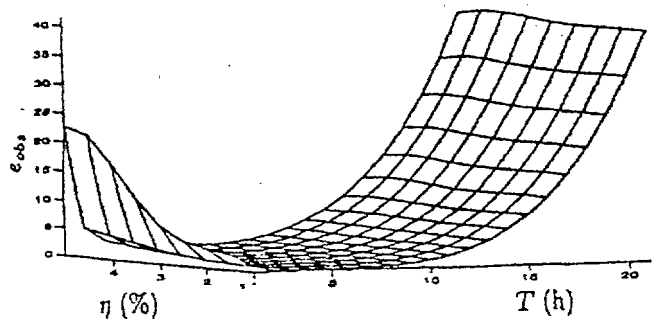

Figure 5. Tuning of the estimation time constant

2. there exists an optimal choice of the time constant which lies around T- 5 (h).

For the control of the process, i.e. regulation of the glucose concentration during the production phase we propose the following adaptive feedback controller:

$$v_{lc} = \frac{V}{C_s^{in} - C_s}\left(\sigma^{calc} C_x + \frac{u}{v} C_s + \lambda\left(C_s^* - C_s\right)\right)$$

$$\sigma^{cafe} = \theta \frac{C_s^* - C_s}{C_x}$$

where σ^{calc} denotes the adaptation parameter, while λ and θ can be considered as the controller gains. The gains λ and θ are tuned as shown in Figure 6 with respect to the relative mean square regulation error. The following conclusions can be drawn from this figure:

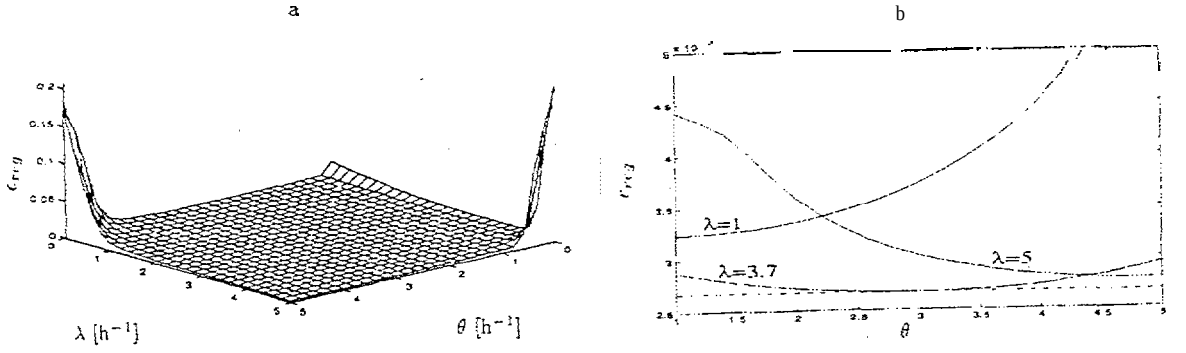

Figure 6 - Tuning of the adaptive feedback controller

1. as expected e_{reg} rapidly increases as λ or θ tends to zero

2. there exists an optimal choice of the gains which lies around $\lambda = 3.7$ and $\theta = 2.9$

4.5 Development of the complete control system

The complete control system installed in the 200 litres fermenter manufactured by SGI is shown in Figure 7.

Either the ceramic probe from SGI or the ABC probe could be inserted in the fermenter. A sampling pump sends the filtrate to the two ETs, one for analysis of glucose and the other for analysis of penicillin. The signals from these thermistors are processed by a LT-CONTROL software for automatic control of a Rheodyne valve through the actuators and it also does penicillin and glucose concentration data acquisition. This software is connected to the MRU penicillin and glucose modules allowing BIOAC visualization and ET calibration of penicillin and glucose. The BIOAC software which contains the UCL peniciHin model software uses these values for the control of the fermentation through the feeding of glucose to the fermenter.

4.6 Industrial project validation

Experimental Set-Up

The ABC microfiltration module was used for aseptic in-situ on-line sampling of the fermentation broth. The filtrate obtained was split in a two channel chamber and passed through two ET columns for penicillin and glucose assaying, as

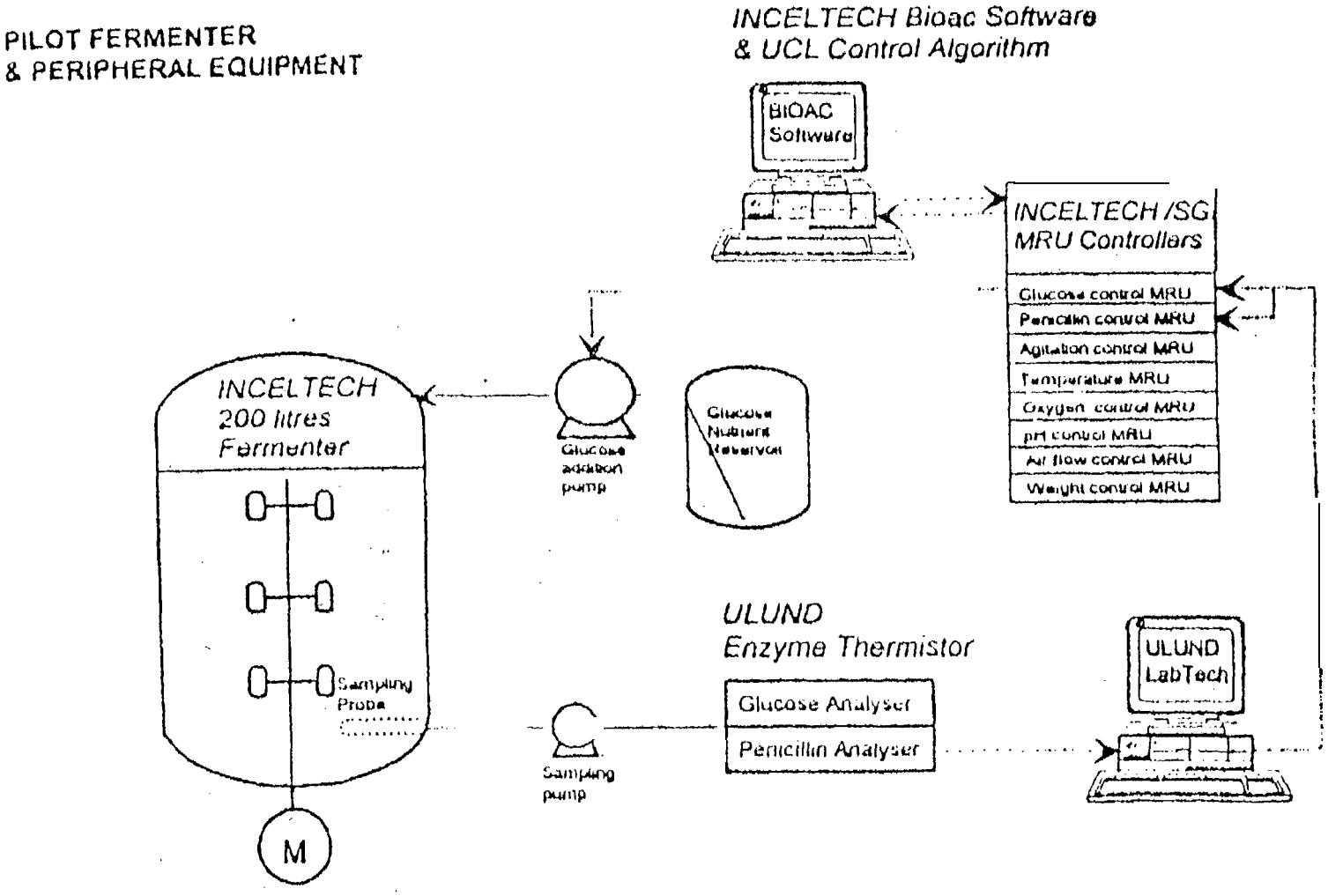

Figure 7 - Bioreactor with on-line analysers and control hardware and software.

described in sub-tasks L 1, 1.3 and 1.4. A sampling frequency less than $1\,h^{-1}$ was thus obtained.

Data acquisition and visualisation was carried out with the MRU/BIOAC software suite installed in the PC connected to the fermenter.

Validation

The proposed optimal operation (sub-task 3.1) was implemented using the adaptive control methodology developed at UCL (sub-tasks 3.2,3.3 and 3.4).

' The dextrose feed rate during the growth phase was adjusted to maintain a constant specific feed rate until the pre-determined optimal glucose amount devoted to biomass growth was exhausted. Maintaining a constant specific feed rate instead of a constant feed rate, ensured that the highest specific growth rate possible in this period was obtained without afecting penicillin production afterwards.

The set-point for the residual glucose concentration in the broth was 0.1 g/l, as proposed earlier in (sub-task 3.3).

Two pilot-plant fermentations were run in parallel at CIPAN, S. A. using similar tanks (e.g., identical geometry, turbines, etc.). The first tank was interfaced with i) the on-line sampling and instrumentation described before and ii) the UCL-BIOAC software (sub-task 3.4), thus having the dextrose feeding automatically controllled. The second tank was operated nominally (i. e., feeding procedure developed in-house) and used as reference. Both tanks were initially charged with the same culture medium and inocculum

The results obtained are presented in Figures 8 and 9, and in Table 4

Table 4 Comparison of Performance Criteria

Index	Controlled run	Reference run	0/0 Improvement
Final titer (g-PENG/*l*)	31.2	27.0	16
Productivity (g-PENG/*h*)	41.5	31.9	30
Product yield (g-PENG/g-glucose)	0.15	0.13	15

Conclusions and Significance of the Optimal-Adaptative Control Policy Evaluated

Table 4 shows significant gains in productivity and considerable gains in terms of final titer, and yield.

Figure 8 shows that the controlled and reference runs differ most significantly in the penicillin and residual glucose concentration profiles, as i mended, producing in the former increased volumes of broth withhigher potency.

Figure 9 shows that the implemented control policy was able to increase significantly the specific production rate in the first half of the fermentation - the period in which the microorganism has the highest producing capacity.

The waving in the glucose concentration in the broth and consequently in penicillin specific production rate, was due to a change in syrup batches having different dextrose contents. The controller was not informed of the change and had some difficulty in acting due to the noise in glucose data.

In view of the results obtained with a controlled dextrose feeding, in terms of criteria used in the process optimization, it is recomended the above mentioned control policy be adopted as a mean to obtain increases in process performance and reproducibility.

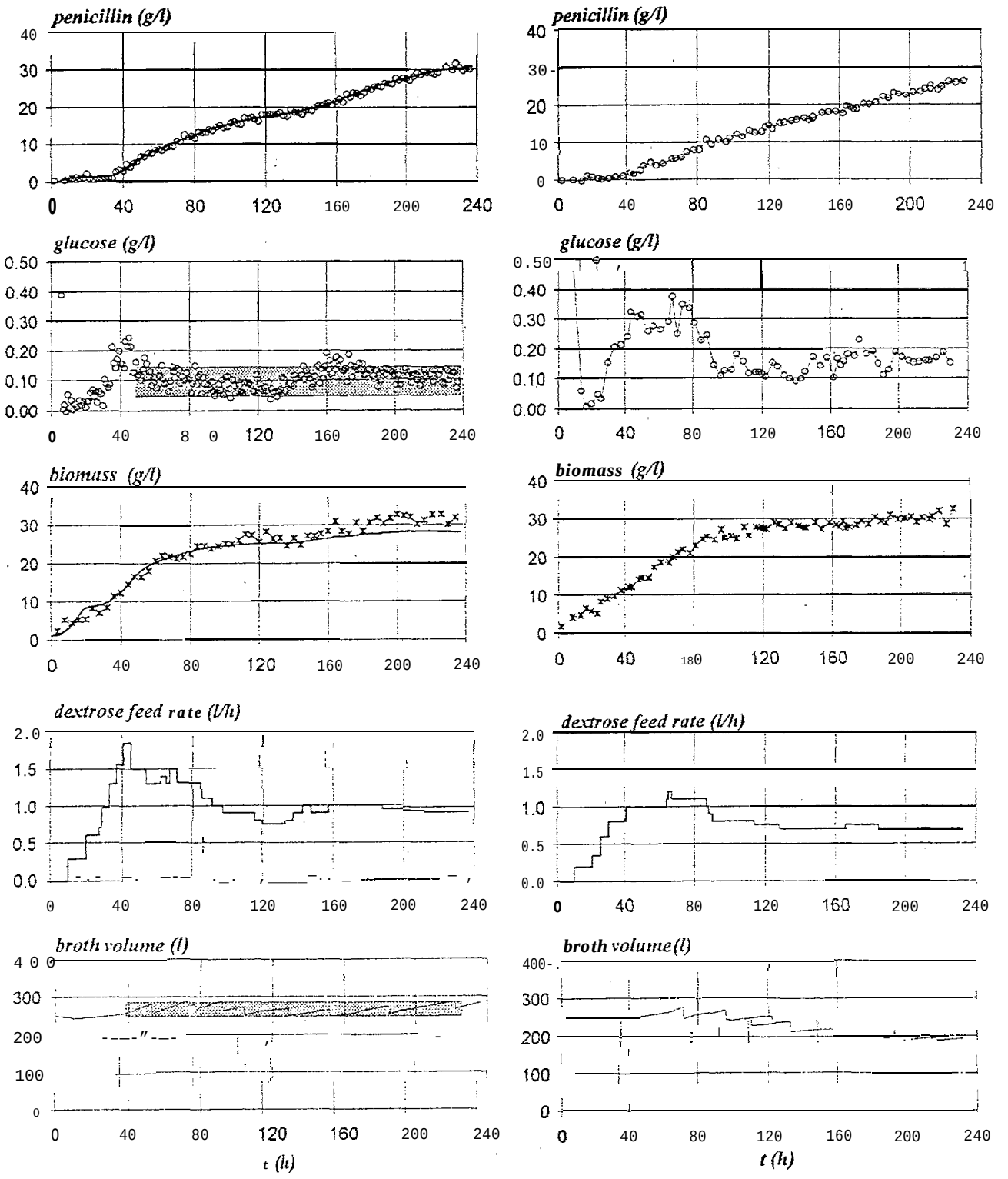

Figure 8 -Experimental validation of the optimal-adaptive control methodology proposed.

Comparison between a fermentation with dextrose feeding under control (*a*) and a reference run operated nominally (*b*).

▒ indicates the period under controlled operation and the setpoint for glucose control (± measurement error @ 95% confidence)

– in *a*) indicate controller and software-sensors outputs.

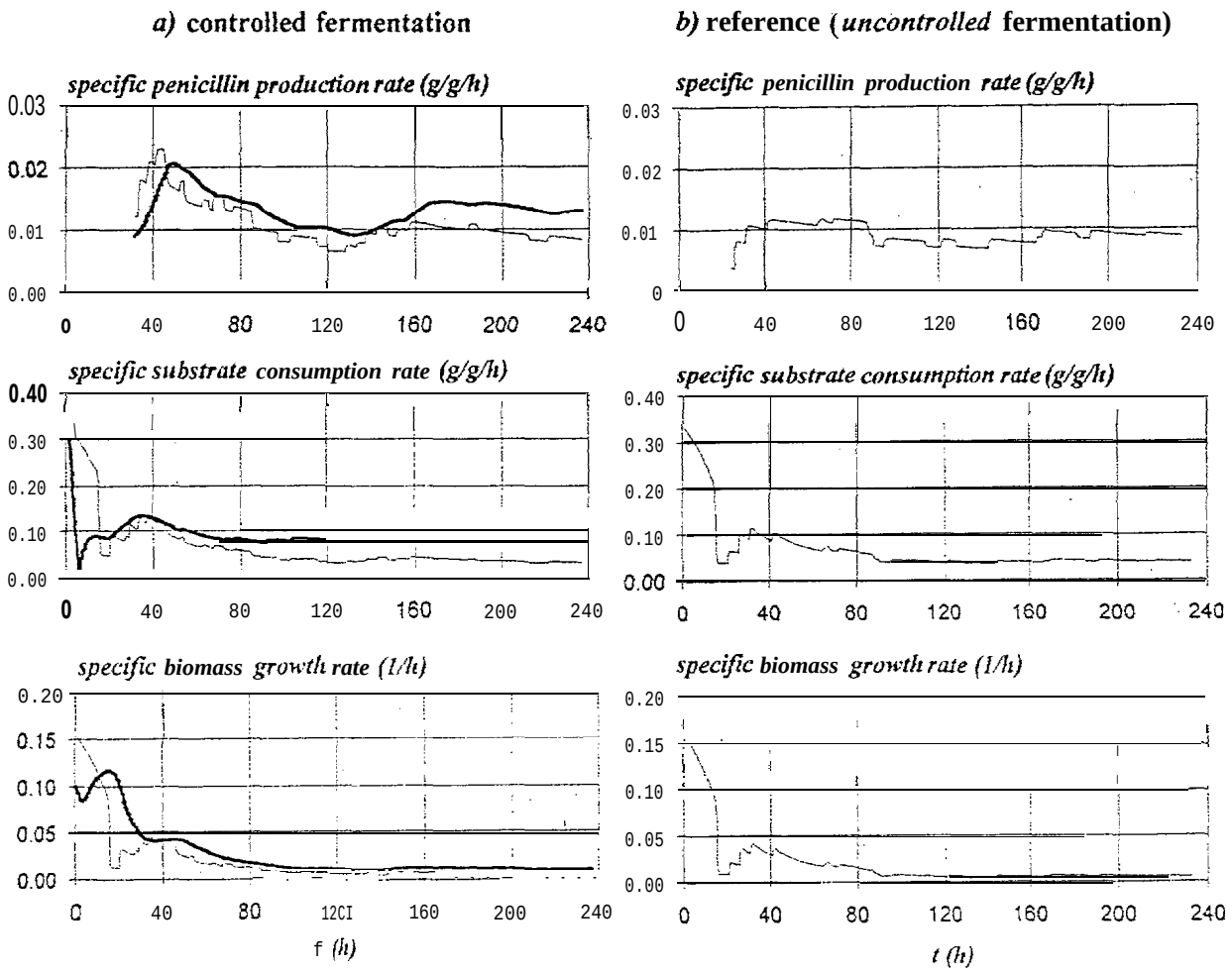

Figure 9 -Experimental validation of the optimal-adaptive control methodology proposed.

Comparison between a fermentation with dextrose feeding under control (*a*) and a reference run operated nominally (*b*).

in *a)* indicate specific rates computed from software-sensors outputs

in *a)* and *b)* indicate specific rates computed from experimental data

5. COMPARISON OF INITIALLY PLANNED Activities AND WORK ACTUALLY ACCOMPLISHED

Table 5 makes the comparison between planned activities and work actually accomplished

Table 5 Comparison between planned activities and work actually accomplished

TASK	PLANNED ACTIVITIES	WORK ACTUALLY ACHIEVED
1.1	Assembling of the thermistor analytical system and application to penicillin and glucose measurement	As planned
1.2	Testing of the thermistor system	As planned
1.3	Design of' an improved on-line automatic sampling device for application to penicillin fermentation	Despite all the work done it was not possible to connect the SAMPLOR to the fermenter, because of the different results obtained compared to manual samples. However, the ceramic probe and the ABC probe could be used connected to a pump.
1.4	Implementation of an on-line sampling acquisition system	As planned, except for the use of automatic sampling device.

1.5	Extension of anlytical capabilities in relation to the true physiological state of the cells	Although methods were developed for analysis of ATP and NADH these methods were not used in the penicillin model developed by UCL.
2.1	Penicillin fermentation model studies	As planned, The model developed could also describe fermentations carried out with a more productive strain not planned to be used in the project
2.2	Simulation of the process dynamics and analysis of structural properties of the mathematical model	As planned
2,3	Design of adaptive state observers	As planned
2.4	Experimental validation	Software sensors have not been validated on-line. An off-line validation has, however, been carried out instead under realistic conditions.
2.5	Extending the use of software sensors to the monitoring of intracellular physiological state marker	None of the results obtained in this sub-task were of interest for the project.
3.1	Formulation of optimization goals in terms of adaptive control	As planned
3.2	Design of adaptive control algorithms	As planned.
3.3	Critical appraisal of control performance and simulation	As planned
3.4	Development and debugging of control software	As planned
3.5	Experimental validation ,	As planned, but not itt representative penicillin fermentations.
4	Industrial pilot validation	As planned, but at the level of a fermenter of 540 litres with 300 litres of broth.

6 LIST OF PUBLICATIONS, CONFERENCE PRESENTATIONS AND PATENTS RESULTING FROM THE PROJECT

i - Demonstrations given:

A demonstration of the ET was made at INETI *in* March 1993 for participants in a Biotechnological Course given by FORBITEC, Associação para a Formação em Biotecnologia

A visit to Pilot Plant at CIPAN, S.A. for demonstration of the SGI sampler system was made during the twelve month project meeting.

2- Publications and Conference presentations:

During the course of this project, the UCL group has published and submitted more thatn twenty papers and communications concerning the modelling and control of biological systems. However, none of them was specifically devoted to penicillin production. Moreover, the discussion on the yield - productivity conflict in penicillin fermentations was presented in a Symposium of ECB 7 (European Congress on Biotechnology, Nice, February i 995), but was not published in the proceedings of the congress. Duarte, J. C. & Pissarra, N. P. "Investigation on penicillin fermentation stoichiomet ry: physiology and modeling", European Laboratory Without Walls, Workshop of Louvain-la-Neuve, in the field of "Advanced monitoring and computer control of Biotechnological Processes", Belgium, September, 1992.

Lageiro M., Barroso C., Mates J., Rebelo L & Duarte J., "Monitoring and Control of Penicillin Fermentation", Poster EB 108 in "Livro de resumes do Biotec 94" at Algarve, Portugal, October 1994 (Poster presentation and discussion at t Second Iberian Biotechnology Congress, Biotec '94).

Duarte J., Barroso C., Mates J., Rebelo L & Lageiro M., "CIn-line analysis monitoring and control of penicillin fermentation", MEP 254, Abstract Book, vol.3, ECB 7, Nice, France, February 1995 (Poster presentation at the 7th European Congress on Biotechnology).

Lageiro M., Mates J., Rebelo L & Duarte J., "Biosensor system for monitoring and control", paper in the Proceedings Book M^{2}SABI'95, vol. 1, Brussels, Belgium, May 1995 (Oral presentation at the First international Simposium on Mathematics Modelization and Simulation in Agriculture rind Bio-industries)

Lageiro M., Barroso C., Mates J & Rebelo L, "Fed-Batch fermentation with *Penicillium chrysogenum*" Postgraduation Course on Biotechnology FORBITEC '93, Xth Module, Lisboa, Portugal, March 1993

Danielson B. & Lageiro M "Biosensors" Postgraduation Course on Biotechnology FORBITEC '93, XIIth Module, Lisboa, Portugal, March1993

Lageiro M., "Simulação em computador de processos biotecnologicos" Postgraduation Course on Biotechnology FORBITEC '93, XIIth Module, Lisboa, Portugal, April1993

Lageiro M., "Biosensor system for monitoring and control. Other perspective", Euro-Mediterranean Postgraduate Course 1995, "Bioreactors and Environmental Apllications *Course*",. CFT, INETI, Lisboa, Portugal, September 1995.

Duarte J., Barroso C & Calhau S., "On-line measurement and control of antibiotics fermentation" Bioreactor Engineering course, Bellaterra (Barcelona), Spain, September, 1994.

Duarte, J. C., Barroso, C., Calhau, S. "Control and Modelling of Biotechnological Processes", Design and Operation of Bioreactors Course, at Ege University Biotechnology Turkey, Med Campus Program of the European Comunity, Turkey, June, 1994.

Lageiro M., " Controlo e optmização de fermentatções", at the WWW address: portugal-virtual.ip.pt/PVirtual (Portuguese magazine at the web), on the Science& Technology subject, November 1995.

3 - Patenteable results

The optimal control strategy for penicillin fermentation processes [hat has been developed in this project could be patentable. [t 'should be a property of the members of the consortium that have effectively contributed to its development.

7 EXPLOITATION OF RESULTS

The optimal control strategy for the penicllin fermentation process as a whole that has been developed in the scope of this project can be patentable. It should be a property of the members of the consortium that have effectively contributed to its development. The exploitation potential for the several partners is described below.

CIPAN, S.A. (Partner 1)

CIPAN, S. A. will try to test the whole system in a plant built in India for 700 tons of Penicillin G Potassium per year (LO fermenters of 100 m^3). If the increase in yield is as that obtained in the validation runs then there is scope for the consortium" to exploit the whole process developed in this project by apllying it to other existing penicillin fermentation plants.

SGI (Partner 2)

A utomatic sampling device:

The sampling device developed in the scope of this project allows to automatically harvest filtered samples. Moreover, it can be connected to the ULUNDETs allowing in this way to perform on-line specific analysis according to the enzyme

used in the ET. However, there is still the need to do small improvements irt the sampling device to overcome the problem of bubbling especially in fungi fermentation. So far only one system was sold.

***Connection** to biosensors:*

The connection of the sampling device to glucose and *penicillin* ETs in the 200 litres fermenter allowed SGI to enter the field of *on-line* analysis. According to ULUND experience it is also possible to analyse many other compounds by changing the enzyme used in the ET.

BIOAC (acquisition and control software):

SGI developed the BIOAC software system which is able to control all the fermentation variables. Moreover, the UCL algorithm for the control of the penicillin fermentation through the addition of glucose could *also* be implemented in the BIOAC system. Up to now about 30 standard versions of this system without the UCL algorithm have been sold by SCH.

INETI [Partner 3)

The development of an integrated strategy for on-line sampling, data-acquisition and adaptive control system is of interest for a number of processes and industries in the area of biotechnology. Marketing and selling of the project "outcome" may therefore be of interest for INETI actual and potential clients in this area. It might be that the software developed for the *process* supervision and data integration may also be of particular commercial interest as well as the equipment integration. In glucose analysis a single mixed enzyme column of the ET was evaluated and could represent an important development for the overall economy of the ET application. INETI as a member of the consortium is entitled to benefit ako from the commercialisation of the whole control system.

UCL (Partner 4)

UCL as a member of the consortium *is entitled* 'to benefit *from* the commercialisation of the whole control system.

ULUND (Partner 5)

ULUND has a high potential in commercializing ETs for different analysis implementation - glucose, penicillin, lactate, ATP, etc. Also, *if* the whole system functions as expected then ULUND, as a member of the consortium, is entitled to benefit from the commercialisation of the whole system.

8. GENERAL CONCLUSIONS

At INETI the ET system of ULUND was adapted for measurement of penicillin and glucose. This task was strongly connected with laboratory scale experiments in the 2, 7, 20 and 200 litres fermenters. These laboratory scale experiments allowed on the one hand to test and to enhance the ET system and the sampling devices and on the other hand to establish and to validate physiological models of the fermentation process and derived software sensors. Experiments were also designed in order to establish the most relevant chemical compounds for characterizing penicillin biosynthesis. The analysis of the results of these experiments provided useful information for the formulation of the control objectives in terms of the regulation of fermentation variables (substrate concentration), A physiological model of' the fermentation process was required to design accurate software sensors and efficient control algorithms. This model-developed by UCL provided *the* ' dynamic behaviour of the most relevant fermentation variables (biomass, penicillin, glucose, dissolved oxygen and -carbon dioxide). The model was elaborated from the results of preliminary laboratory scale experiments at INETI and pilot plant fermentations performed at CIPAN, S. A., from the data reported in the literature and refined according to_the results of specific experiments performed at INETI and CIPAN, S. A..

An automatic sampling device that can be used for fungal penicillin fermentation was designed. Prototypes were tested through INETI laboratory scale experiments and C IPAN, S.A. penicillin fermentations. Software sensors for biomass and penicillin were designed according to the structure and to the mathematical properties of the physiological model.

The results of laboratory scale experiments were used *in* the design *step,* Specific experiments were also designed in order to validate these on-line estimation tools. The physiological model was used to define the control objectives. Adaptive optimal control strategies **were** then proposed according to the set of measured variables (direct on-line measurement or indirect on-line measurement through software sensors). Special attention was paid to penicillin's nature as a secondary metabolite as well as a product of the fermmentation. The control design was strongly interconnected with the results of laboratory scale experiments, The on-line sampling system, the *software sensors* and the adaptive control algorithms were integrated in a complete on-line monitoring and control system for the penicillin fermentation process. This integrated system was tested on the laboratory scale setup and validated at the industrial level and indicated that appreciable *gains* in penicillin fermentation could be obtained. Although not all the objectives of the **project** were achieved, the *results* obtained, as was demonstrated, have high potencial for commercialisation,