Universitat Autònoma de Barcelona

MELISSA collaboration agreement ECT/FG/CB/95.205

ESTEC/CONTRACT 11549/95/N2/FG

- TECHNICAL NOTE 25.330 -

NITRIFYING COMPARTMENT STUDIES

Starting of the nitrifying reactor.

Version : 1
Issue : 1

PEREZ J.; MONTESINOS J.L. ; GODIA F.
Dpt. Enginyeria Química
Universitat Autònoma de Barcelona
08193 Bellaterra, Barcelona, Spain

SEPTEMBER 1996

STARTING OF THE NITRIFYING REACTOR. PHASE 1.2

In the analysis of the physical characteristics of the packed bed reactor that will be used in the nitrifying compartment, there are a number of basic aspects that are considered in this technical note:

- the hydrodynamic flux model in the bioreactor. For different operating conditions the liquid phase in the bioreactor will present different degrees of mixing that will clearly influence the bioreactor performance.

- the mass transfer between different phases. Particularly the oxygen transfer from gas to liquid phase is studied.

- the phase hold-up. In three-phase bioreactors, involving solid, gas and liquid phases, an important feature to know is the percentage of the total volume occupied by the each phase.

These factors are influenced by each other, and also, by the biomass time evolution and rheology of the culture medium. Preliminary studies have been carried out, with the bioreactor without biomass, and selecting the operating conditions following previous work already developed for this reactor (Forler, 1994). Specially for the recirculation ratio (feed flow/recirculation flow) and stirring speed at the reactor inlet. Thus, with the experiments conducted the physical characterization of the reactor have been obtained.

1.- Liquid-phase Mixing.

The degree of mixing in reactors can be measured using RTD (residence time distribution) analysis. This information can be determined by the stimulus-response technique. The method involves the introduction of a tracer material at the inlet or some other point within the reactor, and the observation of the subsequent response in the effluent stream or some other downstream point within the reactor. The distribution of residence time is obtained from the response and a suitable model for the flow can then be selected by matching the experimental response with that obtained from the mathematical model

(Levenspiel, 1972).

Various tracer substances may be used, provided that they satisfy the following conditions: they must be soluble in one phase only, a sensitive detection method and equipment with a high time resolution capacity are available, and trace products are inert (no chemical reaction with the phase and no adverse effect on biological processes).

The use of dyes provides a valuable visual marker of the tracer's presence; however, colorimetric detector can give nonlinear responses at low concentrations. This complicates the measurement of the complete experimental RTD curve, especially if there is considerable tailing due to the presence of regions of static liquid hold-up (Swaine et al. 1988).

Step or pulse functions, or occasionally harmonic or stochastic functions, are used as test signals. The test function amplitude spectra show that the ideal delta function and the ideal stochastic test signal provide the most information. In practice, pulse, imperfect pulse or step inputs of tracers are most commonly used.

1.1. Experimental procedure.

A blue dextran dye was used as tracer. It has a high molecular weight ($2*10^6$) avoiding its entry inside the polystyrene beads. This compound is easy to detect and analyze by absorbance measurement at 618 nm.

In the experiments, a pulse injection was done at the inlet of the reactor (central position) and samples were taken at the outlet of the reactor (central position). The trace volume and time for injection employed have been low enough to consider them as a ideal pulse for practical purposes (volume of tracer = 10mL, in respect to a reactor volume = 3800 mL).

Due to the fact that the Nitrifying compartment has been designed to work at high liquid recirculation rate and important gas flow-rate (Forler, 1994), it has been taken as a first work hypothesis that the flow of the reactor could be represented as a well-mixed tank in a good approximation. But, it should be taken in mind the possible existence of an important mixing time, different from zero, due to the reactor configuration. It could have strong consequences in the control and reactor operation. Thus, the equation that is applied to determine the mean residence time is as follows:

$$C = C_0 \exp(-\frac{t}{\theta}) \qquad (1.1)$$

Then, the parameter evaluation was done by least-squares curve fitting based on the minimization of the sum of squares of the differences between the experimentally measured response curve and curves predicted by the linearized flux model investigated:

$$\ln C = \ln C_0 - \frac{t}{\theta} \qquad (1.2)$$

1.2. Results of the RTD analysis.

The calibration curve corresponding to different concentrations of blue dextran is presented in **Figure 1.1**. It fits well throughout the concentration working range: [0.0 g/L - 0.25 g/L]. Thus, the following calibration equation is obtained:

$$A = 0.7950 * C + 0.0067 \qquad R = 0.9995 \qquad (1.3)$$

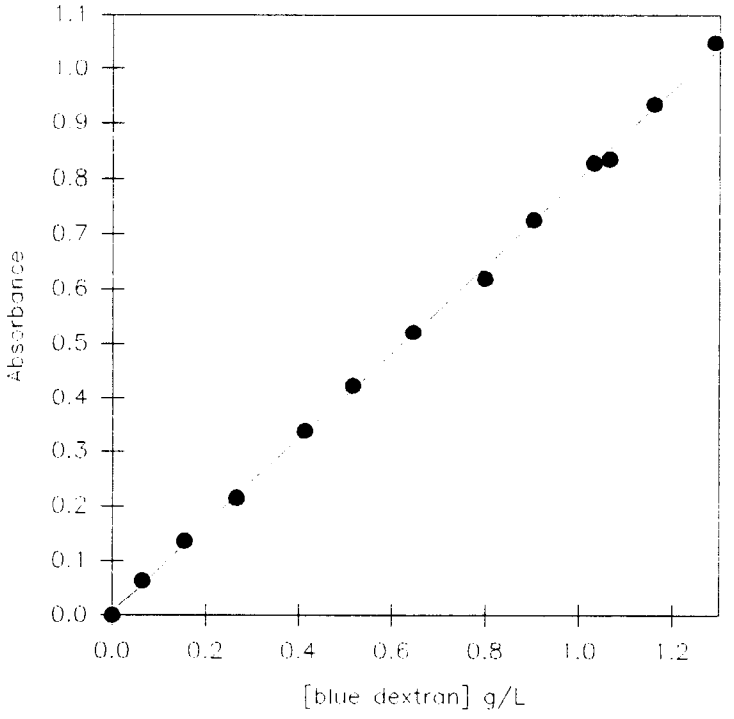

Figure 1.1. Calibration curve of the blue dextran dye concentration.

The experiments were conducted with two different recirculation flows 18 ml/min and 45 mL/min. The rest of the operating conditions were the same for three experiments, considered as a conservative values, as stated in previous work :

Air gas flow= 3 L/min (open loop)
Stirring rate = 400 rpm
Liquid input flow = 2.8 mL/min

Results for the concentration-time profiles (normalized curves,see NOTE 1) obtained by the tracer pulse injection and the determination of the mean residence time by linearization of the flux model equation are presented in **figures 1.2, 1.3, 1.4 ,1.5 ,1.6 ,1.7 .** It can be appreciate clearly that the consideration of a rather simple mixed tank is appropriate.

The mean residence times obtained in the experiments were:

Experiment 1, with recirculation flow = 45 mL/min (mass of the tracer injected = 0.57g of blue dextran, injection volume = 10mL).
Residence time = 24.0 h (regression coefficient = 0.999), that agrees with the data obtained from the experiments performed to determine the gas hold-up, and physical dimensions and characteristics of the reactor.

NOTE 1 : It can be obtained the normalized curve with this equation (Levenspiel, 1972):

Et = (liquid input flow)*[concentration of tracer]/(mass of the tracer injected)

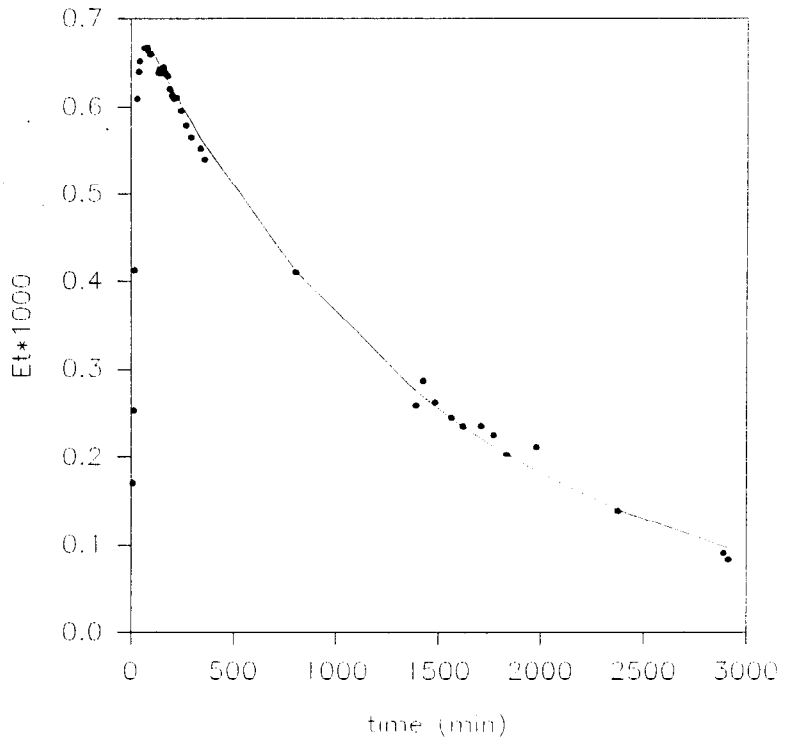

Figure 1.2. Normalized curve of the residence time distribution. (rec. flow = 45 mL/min)

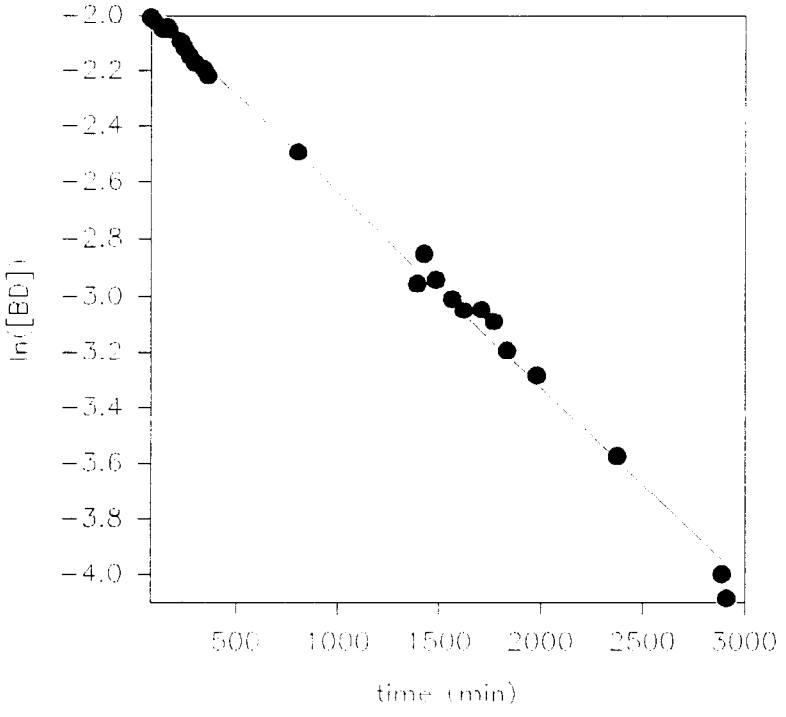

Figure 1.3. Results from the linearization of the flux model equation (rec. flow = 45 mL/min)

Experiment 2, with recirculation flow = 18 mL/min (mass of tracer injected = 1 g of blue dextran, injected volume = 10 mL).

Residence time = 24.1 h (regression coefficient 0.999).

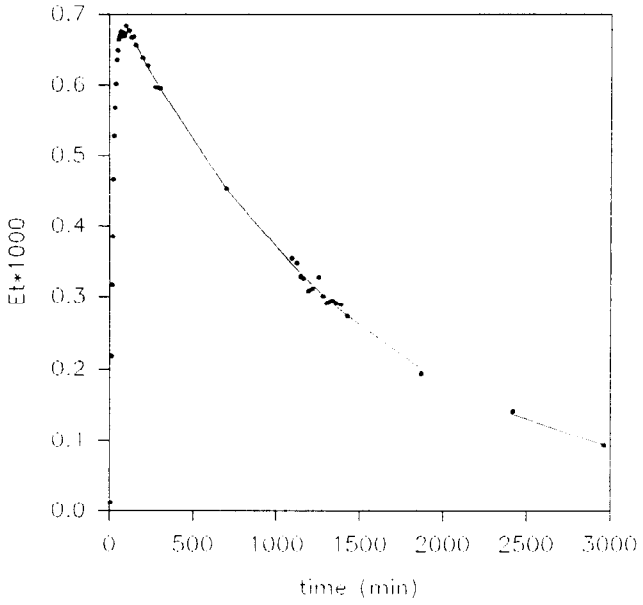

Figure 1.4. Normalized curve of the residence time distribution. (rec. flow = 18 mL/min).

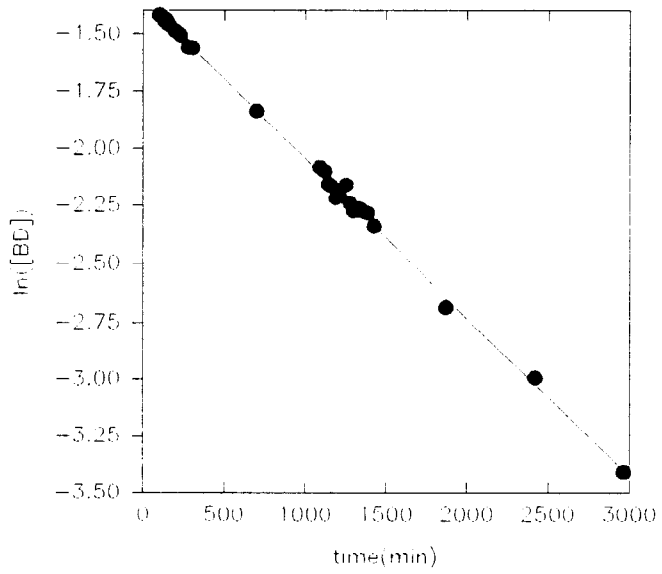

Figure 1.5. Results from the linearization of the flux model equation. (rec. flow = 18 mL/min)

Experiment 3, with recirculation flow = 45 mL/min (mass of tracer injected = 1g of blue dextran, injected volume = 10 mL).

Residence time = 24.1 h (regression coefficient = 0.9999)

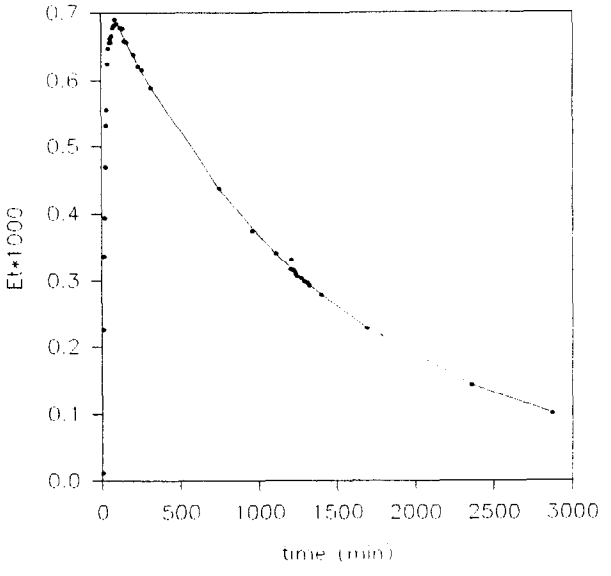

Figure 1.6. Normalized curve of the residence time distribution. (rec. flow = 45 mL/min)

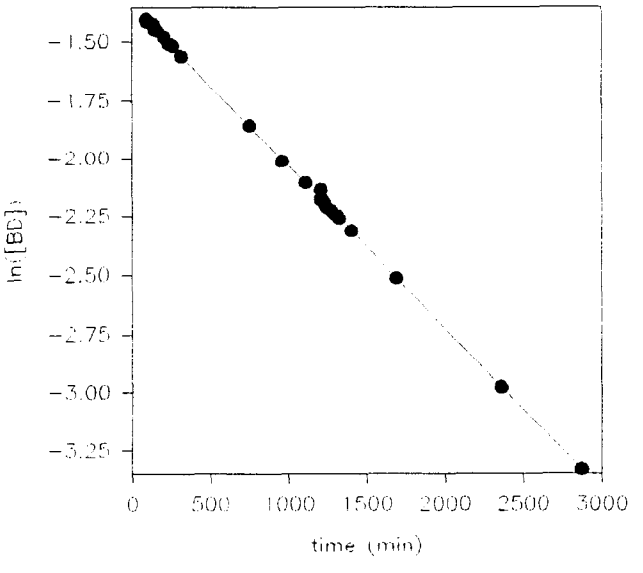

Figure 1.7. Results of the linearization of the flux model equation. (rec. flow = 45 mL/min)

NOTE 2: the data corresponding to these experiments are presented as an appendix to this T.N.

The last test of this set of experiments was done with the aim of elucidate what was the effect of liquid recirculation in the system. Thus, no liquid recirculation was employed. Results form experiment 4 can be seen in figure 1.8 and 1.9 showing only slight differences related to the one with liquid recirculation.

Experiment 4, without liquid recirculation (mass of tracer injected = 1g of blue dextran, injected volume = 10 mL). Residence time = 24.1 h (regr. coeff. = 0.997)

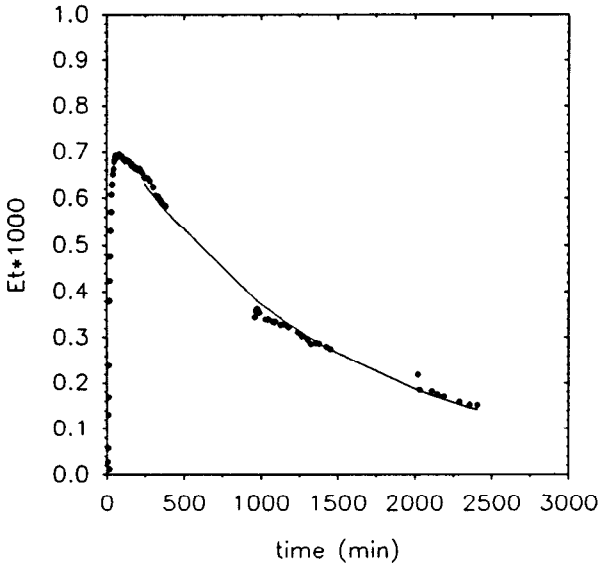

Figure 1.8. Normalized curve of the residence time distribution. (without liquid recirculation)

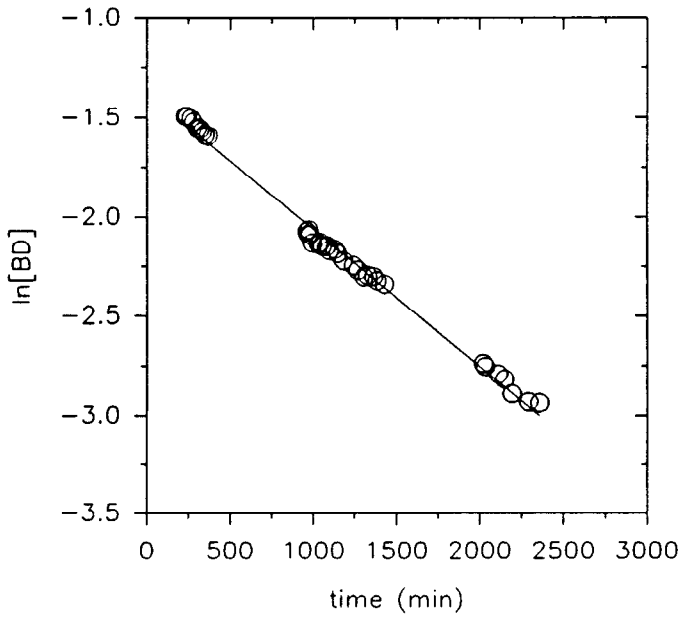

Figure 1.9. Results of the linearization of the flux model equation. (without liquid recirculation)

In order to avoid possible mixing effects due to the way of the tracer was injected, e.g. from the bottom section to the active area of the reactor, another set of experiments were carried out. In this new set of experiments the tracer was injected from the top of the reactor to the active area by means of a long and narrow glass tube.

In experiment 5 liquid recirculation was not used, to eliminate the mixing effects of the agitated section at the reactor inlet.

The conditions for this experiment were: **Experiment 5, without liquid recirculation (mass of tracer injected = 1 g of blue dextran, injected volume = 10 mL).**
The air flow-rate, stirring rate and liquid input flow were kept at the same values that for the earlier experiments. Results are presented in figure 1.10.

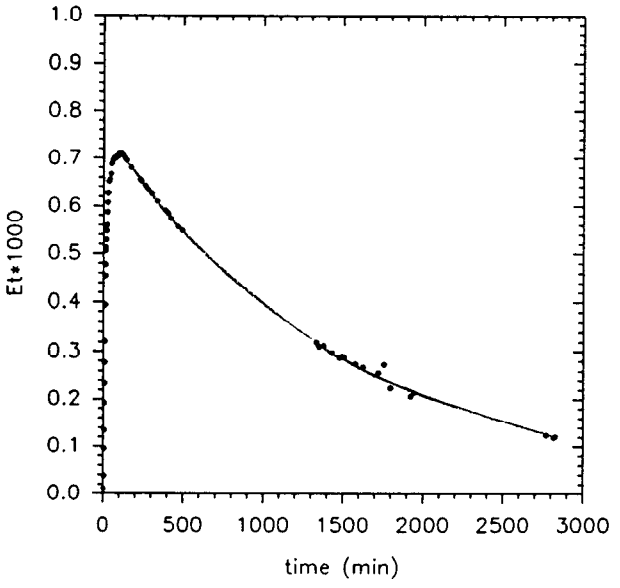

Figure 1.10. Normalized curve of the residence time distribution. (without liquid recirculation)

From the data obtained, it can be concluded that not important changes in the flow pattern are produced when no liquid recirculation is being used keeping gas circulation throughout the reactor.

Another important conclusion that can be obtained is that the mixing of the reactor is accomplished mainly by the effect of the aeration, that is to say, gas circulation throughout the fixed-bed. To corroborate this, an additional experiment were conducted listed Experiment 6.

Experiment 6, neither gas circulation nor liquid circulation is used (mass of tracer injected = 1 g of blue dextran, injected volume = 10 mL).

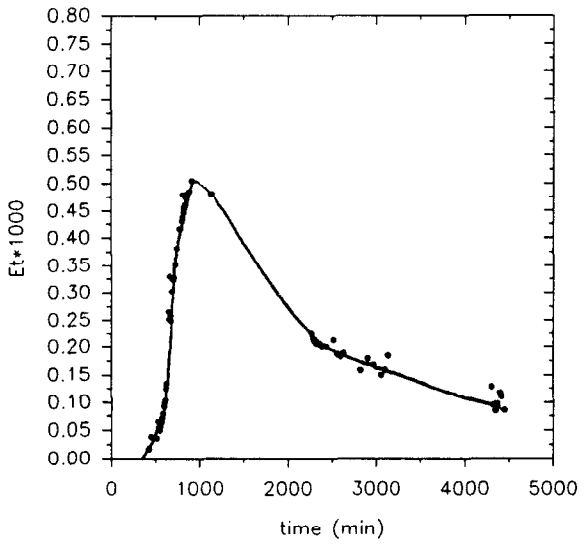

Figure 1.11. Normalized curve of the residence time distribution. (neither gas circulation nor liquid recirculation)

In the experiment 6 without neither gas circulation nor liquid recirculation it can be observed that response curve is clearly composed by two well-known effects (Levenspiel, 1972).

First, the delay of the leading section of the RTD curve. This fits with the existence of an important contribution of moderate-dispersed plug-flow in the system comparing with experiments 1, 2, 3, 4 and 5.

Second, an increasing of the slope for the tailing section of the RTD curve. This corresponds to a decreasing of the effective well-mixed volume.

From this experiment it can be concluded that the presence of gas flow increases the mixing of the system up to become a rather well-mixed system as stated initially.

As a conclusion from this wide set of experiments, it can be pointed out that the mixing of the bioreactor is mainly caused by the high gas circulation flow. Only a little

contribution on mixing is achieved by means of liquid recirculation. It may vary upon lower air gas flow was employed. Moreover, it must be bear in mind that the biomass and rheology of the medium culture may affect the flow pattern of the bioreactor system, when the nitrifying compartment will be running.

2. Gas-liquid oxygen transfer.

In aerobic bioreactors, sufficient levels of oxygen must be supplied to the liquid broth to establish optimum conditions for microbial growth. Often, the oxygen transfer rate is the limiting factor of a bioreactor system and an important parameter for scale-up. The oxygen transfer rate is dependent upon the volumetric mass transfer coefficient k_La, and the driving force, $(C^* - C)$ (where C is the dissolved oxygen concentration in the liquid phase and C^* is the oxygen saturation concentration in the liquid phase at the gas-liquid interface) (Dunn et al., 1975).

The oxygen transfer rate can be measured by chemical or physical techniques. The application of a chemical technique requires accurate knowledge of the reaction kinetics that can be difficult to determine. A physical technique usually involves performing an step-up or step-down in the oxygen concentration of the bioreactor inlet gas and measuring the dissolved oxygen concentration. Steady state is first established with either air or nitrogen sparging and either oxygen or nitrogen is then substituted by air or nitrogen. A variant of this method is the dynamic pressure method, where the pressure inside the bioreactor is increased causing an instantaneous increase in the interfacial oxygen concentration. Although these physical methods can be applied to bioreactor systems, they require either an independent measurement of the volumetric oxygen uptake rate by the microorganisms in the bioreactor or an accurate determination of the interfacial oxygen concentration (van't Riet, 1979).

The physical "gas out-gas in" is another dynamic method that avoids these problems by determining directly the volumetric mass transfer coefficient in the actual microbial broth. It is based on the measurement of the dissolved oxygen concentration in the medium. If the gas supply to the bioreactor is turned off the dissolved oxygen concentration will decrease at a rate equal to the oxygen consumption by the bioreaction. From measurement of the dissolved oxygen the volumetric oxygen consumption rate can be determined (if the rate is

independent of C the dissolved oxygen concentration decreases as a linear function of time). When the gas supply is turned on again the dissolved oxygen concentration will increase back to the initial level, and by using the estimated (average) value for oxygen consumption rate, the value of k_La can be determined from the measured profile of dissolved oxygen (van't Riet, 1979).

For all the physical methods the determination of the oxygen mass transfer coefficient becomes inaccurate when the response time constant (τ), of the dissolved oxygen probe is large. The response time constant is defined as the time at shich the probe reaches 63.2% of its final value when exposed to a step change in concentration. For accurate values, a criterion of $\tau < (1/k_La)$ is usually recommended (van't Riet et al., 1991). The commercially available sterilizable dissolved oxygen probes which are used for measurements in bioreactor systems have large response time constants of 10-100s. Several models for the probe response have been developed, but most modern sterilizable probes can be approximated by a first-order system.

2.1. Experimental procedure.

The dynamic method can also be applied for conditions where there is no reaction. This is interesting when studying the influence of operating parameters, e.g. the stirring speed, gas flow rate, recirculation ratio and so on, on the volumetric mass transfer coefficient. Hence, we decided to use this method because of its simplicity, but considering the dynamics of the probe. The probe response may be described a first-order deconvolution relationship for the liquid phase:

$$\frac{dC_{med}}{dt} = \frac{(C - C_{med})}{\tau} \qquad (2.1)$$

Data obtained for the response of the two sterilizable electrodes from Ingold Electronics to a step in dissolved oxygen concentration (not in the reactor) verified the applicability of the first-order system to the response of such a probe, as can be seen in **Figure 2.1**. The electrodes were calibrated before each experiment using a highly agitated, oxygen-saturated sample of water (which had been sparged with air). This type of probe gave

a response time of approximately 25 s. Ingold lists 98% response in 60 s, which, for a first-order system, corresponds to a response time constant of 15 s based on 63.2% response (Cole-Parmer Inst.). The discrepancy may be due to the aging of the membrane. A first-order system with a response time of 25 s was assumed to minimize the errors caused by the probe response.

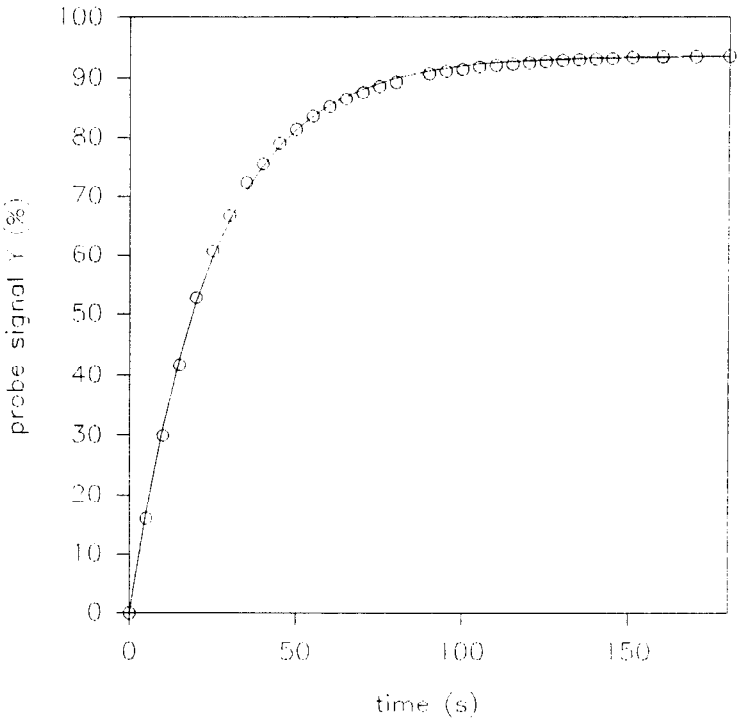

Figure 2.1. Response of the oxygen electrode to a step concentration

After the probe characterization, the determinations in the packed-bed bioreactor were carried out. First, the oxygen content in the tap water (liquid phase) of the reactor was purged using nitrogen until the oxygen concentration in the water was reduced to a steady state value of approximately zero. Then, at steady fluid temperature a small air flow was introduced, producing an increase of dissolved oxygen concentration until a new steady state was reached. The fixed-bed reactor is equipped with two oxygen electrodes, one at the top and the other at the bottom. The time-change in the oxygen dissolved concentration was continuously monitored at this two points from the instant at which the air-flow began.

The rate of oxygen transfer is related to the overall volumetric mass transfer coefficient (k_La) and the concentration driving force by the equation:

$$\frac{dC}{dt} = k_La\ (C^* - C) \qquad (2.2)$$

where C and C^* are the instantaneous and the saturation (or equilibrium) concentration,

respectively, of the dissolved oxygen in the liquid. For steady hydrodynamic and physicochemical conditions the k_La coefficient is time-invariant. This procedure assumed a constant gas-phase composition, "well-mixed" liquid and that the effect of the beads is negligible (in terms of oxygen transport). For practical purposes, the oxygen partial pressure in the gas-phase remains unchanged and the assumption of a constant gas phase composition applies. In the same way, the saturation value of oxygen in the liquid is considered constant, despite the variations in the hydrostatic pressure (Chisti, M.Y., 1989). As we have discussed previously, the fixed-bed reactor can be considered approximately well-mixed in the liquid phase, at the experimental conditions used in these tests.

The theoretical signal, at time t, was obtained by convolution of the concentration history from the start of the experiment at initial concentration C_0, until time t with the probe response characteristics (from eq. 2.1 and 2.2) (Chisti, M.Y., 1989):

$$C_{med} = C^* + \frac{C^*-C_0}{1-\tau\ k_La}\ (\tau\ k_La\ \exp(-\frac{t}{\tau})\ -\ \exp(-k_La\ t)) \qquad (2.3)$$

2.2. Results of the k_La determination.

The k_La coefficient was determined by curve-fitting of the experimental data to the theoretical oxygen concentration time-profiles, using a non-linear procedure.
An example of the k_La determination for a given conditions is presented in **Figure 2.2,** showing a good concordance between theoretical and experimental data.

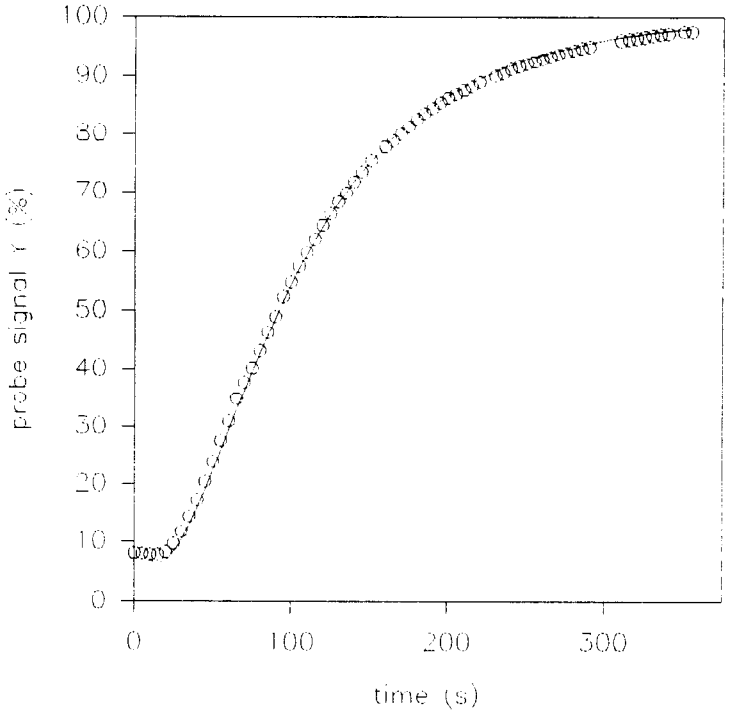

Figure 2.2. K_La determination. Experimental probe signal (%) and its theoretical profile.

The influence of aeration rate, stirring speed in the stirred inlet section and recirculation ratio on k_La was examined. Data for the fixed-bed reactor top and bottom sections are summarized in **Table 2.1.** All the experiments have been conducted with an input liquid flow-rate of 2.8 ml/min taken as a mean operation value for this reactor as stated in previous work (Forler, 1994).

The k_La coefficient increases with increasing gas aeration rate and with increasing stirring speed in the reactor inlet section. Because of the high recirculation ratios applied (based on a well-mixing criteria) no effect was observed by changing these ratios. In general,

the k_La is greater for the bottom section than for the top section. Only slight differences were observed at high air flow rates between the top and bottom sections. Typical k_La values for stirred tanks range from 0.002 to 0.8 s^{-1} (Schügerl, 1991).

Reactor Section	Air Flow (l/min)	Stirring (rpm)	Rec. flow (ml/min)	Rec. ratio (Rec/Input)	k_La (s^{-1})
TOP	3.0	400	18.0	6.0	0.014
TOP	3.0	400	45.0	15.0	0.014
TOP	3.0	200	18.0	6.0	0.014
TOP	5.0	400	18.0	6.0	0.023
BOTTOM	3.0	400	18.0	6.0	0.018
BOTTOM	3.0	400	45.0	15.0	0.018
BOTTOM	3.0	200	45.0	15.0	0.013
BOTTOM	5.0	400	18.0	6.0	0.024

Table 2.1. Effect of aeration, stirring speed and recirculation ratio on the k_La coefficient for the top and bottom sections of the fixed-bed reactor.

Thus, the k_La values obtained ranging from 0.01 to 0.03 s^{-1} are considered as satisfactory due to the well-known low oxygen transfer efficiency of this kind of reactors which are not completely mechanically stirred (tubular loop reactors 0.02-0.2 s^{-1}, bubble column 0.02-0.8 s^{-1} (with ethanol) (Schügerl, 1991). However, not only the oxygen transport in the liquid phase must be taken into account, if it is wanted not to have oxygen limitations for the microorganisms. It will be necessary to consider the oxygen transport through the biofilm and/or inside the beads, because normally it can be the limiting step of the process.

3. Gas hold-up, solid, liquid and gas volume determination.

One of the hydrodynamic parameters of interest for the characterization of the fixed-bed is the overall gas hold-up. The gas hold-up or the gas void fraction is defined as the volume fraction of gas-phase for the different phases in the reactor. Gas hold-up increases with increasing gas inlet flow-rate. The importance of gas hold-up is multifold. The hold-up determines the residence time of the gas in liquid and it influences the gas-liquid interfacial area available for mass transfer (Schügerl, 1981). An important phenomena in packed-bed when they are aerated by direct sparging is the coalescence of the gas phase. The commonly used techniques of gas hold-up measurements have been reviewed by some authors (Merchuck, 1986).

3.1. Experimental procedure

The overall gas hold-up was determined by using the volume expansion technique, because of its simplicity (and reliability). In the volume expansion method, the measurement of the unaerated, static, liquid with beads volume and the volume upon aeration is used to calculate the overall gas hold-up. The determination of beads (solid) volume was also performed by volume expansion introducing the beads into liquid.

The operating conditions for these determinations were fixed at the following values.

Air gas flow= 3 L/min (open loop)

Stirring rate = 400 rpm

Liquid input flow = 2.8 mL/min

Liquid recirculation flow = 18 mL/min.

3.2. Results of volume determinations

The measurements obtained using the above described methodology were:

Liquid volume = 3800 mL

Solid volume =3900 mL

Gas volume = 400 mL

Gas hold-up = Gas volume /(Gas volume+Liquid volume) = 0.095

Gas hold-up* = Gas volume /(Reactor volume) = 0.049

In **Figure 3.1.** the physical dimensions of the fixed-bed reactor are given.

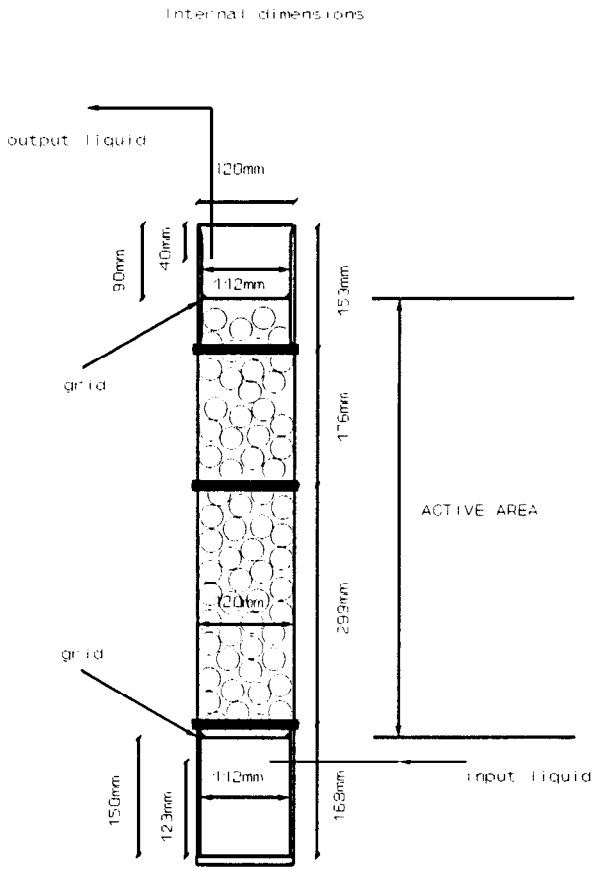

Figure 3.1. General scheme and internal dimensions of the fixed-packed bed reactor.

4. Estimation of the amount of inoculum necessary to start pilot bioreactor

The level of inoculum necessary to start the culture has been estimated from the bioreactor properties, the information obtained in free cell cultures, immobilized cultures and the previous studies published on this compartment (Zehgal 1992, Walter 1993, Forler 1994)

The calculations and considerations are as follows:

Fixed-bed liquid volume = 3800 mL

Mean bead diameter = 4.1 mm = D_p

Surface/volume ratio = $6/D_p$ = A/V

Normal range of required inoculum = 1%-10% of final desired biomass concentration.

Typical final biomass concentration = 10^8 c.f.u./mL (*Nitrosomonas or Nitrobacter* free culture) (Laanbroek, H.J., 1993).

Typical final biomass concentration = 13.33 g dry weight/m^2 (*Nitrosomonas* and *Nitrobacter* fixed biomass co-culture) (Garrido, J.M., 1996)

With these data, the requeriments for the inoculation procedure can be estimated.

$X_{\text{fixed bed min}} = 0.01 * X_{\text{typical}}$ (Initial value for fixed-bed (1%))

$X_{\text{fixed bed min}} = 0.10 * X_{\text{typical}}$ (Initial value for fixed-bed (10%))

Where X is biomass concentration in c.f.u/mL or g dry weight/L.

To calculate the amount of biomass to be inoculated it must be taken into account the total liquid volume of the bioreactor:

Biomass inoculum = $X_{\text{fixed bed}} * V_{\text{fixed bed}}$

So, if it is wanted to give the values in c.f.u./mL:

$X_{\text{fixed bed min}} = 0.01 * 2 * 10^8$ c.f.u./mL = $\underline{2*10^6 \text{ cfu/mL}}$

Biomass inoculum (1%) = $2*10^6 * 3800 = \underline{7.6*10^9 \text{ cfu.}}$

$X_{\text{fixed bed min}} = 0.10 * 2 * 10^8$ c.f.u./mL = $\underline{20*10^6 \text{ cfu/mL}}$

Biomass inoculum (10%) = $20*10^6 * 3800 = \underline{7.6*10^{10} \text{ cfu.}}$

If the values are given in dry weight the $X_{typical}$ must be expressed in g/mL culture.

$X_{typical}$ = 13.33 g/m^2 * (6/(4.1*10^{-3}))* m^2/m^3 * 10^{-6} m^3/mL = 1.95*10^{-2} g/mL bead.

$X_{typical}$ = 1.95*10^{-2} g/mL bead * 3900 mL bead/3800 mL culture medium = 2.0*10^{-2} g/mL

$X_{fixed\ bed\ min}$ = 0.01 * 20.0 g/L= 0.2 g/L

Biomass inoculum (1%) = 0.20 * 3.800 = 0.76 g

$X_{fixed\ bed\ min}$ = 0.10 * 20.0 g/L= 2.0 g/L

Biomass inoculum (10%) = 2.0 * 3.800 = 7.6 g

Due to the low velocity of growth for the *Nitrosomonas* and *Nitrobacter* culture, the proposal for the packed bed inoculation would be to use a value more close to 1-2 % , that is 0.76-1.5 g of cells. These concentrations could be obtained by a culture of 1 L volume of each strain.

5. Proposal of experiments for the next phase and start-up of packed bed bioreactor.

To complete the phase corresponding to the starting of the nitrifying reactor, a test procedure for the continuous experiments to carry out in this compartment in the next phase has been prepared. Also, the data necessary to collect from these continuous experiments has been discussed, evaluated and selected.

The proposed strategy in order to start-up the packed bed bioreactor is to inoculate the bioreactor, after proper sterilization procedure (Forler 1994), with enough amount of biomass, as estimated above, and then in batch mode keep the suitable conditions for growth and biofilm proliferation. The operation conditions will be similar as described in previous work. That is to say, gas circulation (3 L/min), liquid recirculation (18 mL/min), temperature (28 °C), pH = 8, dissolved oxygen (80 %) and culture medium.

When enough biomass will have proliferated covering the beads (biofilm formation) and a reasonable conversion for ammonium will be observed, the continuous start-up will be conducted. First, with low liquid input flow-rate, and after that, step by step increasing the ammonium load to the system.

Along all the start-up procedure and after that, the variables considered as key parameters for the system will have to be measured and registered. These variables includes pH, dissolved oxygen, temperature, ammonium concentration, nitrate and nitrite concentration, flow-rates.

It should be stated that, in view of the different literature reports (Garrido 1996, Zeghal 1992, Walter 1993), the time necessary to reach the biofilm development and start the continuous operation of the reactor will be long, in the order of 3-4 months. After this period, once biofilm would be formed, it is expected to achieve reasonable velocities of ammonia conversion.

REFERENCES

CHISTI M.Y.,(1989) Air-lift bioreactors. Elsevier, New York.

COLE-PARMER Instrument Company Catalogue, 1993-94. Niles, IL.

DUNN I.J., EINSELE A.,(1985), J. Appl. Chem. Biotechnol., **25**, 707-720. Oxygen transfer coefficients by the dynamic method.

FORLER C., (1994), MELISSA: Development of a fixed bed pilot reactor for a continuous axenic coculture of *Nitrosomonas europae* AND *Nitrobacter winogradsky*. YGT ESA/YCL, X-997, 1994.

GARRIDO, J.M., (1996), Ph.D., Nitrificación e desnitrificación de aguas residuales con centracións elevadas de formaldehido e urea. Universidad de Santiago de Compostela.

LAANBROEK, H.J., GERARDS, S., (1993). Arch. Microbiol. **159**: 453-459. Competition for limiting amounts of oxygen between *Nitrosomonas europea* and *Nitrobacter winogradskyi* grown in mixed continuous cultures.

LEVENSPIEL O., (1972), Chemical Reaction Engineering, 2nd. ed., Wiley, New York.

MERCHUK J. C., (1986), in Encyclopedia of Fluid Mechanics, (Cheremisinoff, N.P., editor), **3**, Gulf Publishing (Houston), pp. 1485-1511. Hydrodynamics and hold-up in air-lift reactors.

SCHÜGERL K., (1981), Adv. Biochem. Eng., **19**, 71-174. Oxygen transport into highly viscous media.

SCHÜGERL K., (1991), Bioreaction Engineering, volume 2, Wiley, New York.

SWAINE E., Daugulis, A. J., (1988), Biotech. Progr., **4**, 3, 134-148. Review of Liquid Mixing in Packed Bed Biological Reactors.

VAN'T RIET K., (1979), Ind. Eng. Chem. Process Des. Dev., **18** (3), 357-364. Review of measuring methods and results in nonviscous gas-liquid mass transfer in stirred vessels.

VAN'T RIET K., TRAMPER J., (1991), Basic bioreactor design. Marcel Dekker, New York.

APPENDIX

EXPERIMENT 1 (rec. flow 45 mL/min)

time (min)	[BD] g/L	Et	1000 Et
10	0.0344	0.00017076	0.17076
15	0.051	0.00025317	0.25317
20	0.0831	0.00041251	0.41251
32	0.1229	0.00061008	0.61008
40	0.1289	0.00063986	0.63986
45	0.1312	0.00065128	0.65128
68	0.1343	0.00066667	0.66667
75	0.1341	0.00066568	0.66568
80	0.1344	0.00066717	0.66717
85	0.1334	0.0006622	0.6622
95	0.1327	0.00065873	0.65873
135	0.1287	0.00063887	0.63887
140	0.1294	0.00064235	0.64235
150	0.1285	0.00063788	0.63788
155	0.1294	0.00064235	0.64235
160	0.1299	0.00064483	0.64483
165	0.1289	0.00063986	0.63986
170	0.1284	0.00063738	0.63738
180	0.1279	0.0006349	0.6349
190	0.125	0.00062051	0.62051
200	0.1237	0.00061405	0.61405
210	0.1229	0.00061008	0.61008
225	0.1231	0.00061107	0.61107
245	0.1201	0.00059618	0.59618
270	0.1166	0.00057881	0.57881
295	0.1139	0.0005654	0.5654
340	0.1114	0.00055299	0.55299
360	0.1089	0.00054058	0.54058
803	0.08271	0.00041058	0.41058
1392	0.05218	0.00025902	0.25902
1426	0.05784	0.00028712	0.28712
1485	0.05293	0.00026275	0.26275
1564	0.04941	0.00024527	0.24527
1621	0.0474	0.0002353	0.2353
1709	0.04752	0.00023589	0.23589
1770	0.04551	0.00022591	0.22591
1835	0.04098	0.00020343	0.20343
1980	0.04274	0.00021216	0.21216
2376	0.02801	0.00013904	0.13904
2889	0.01834	9.1041e-005	0.091041
2912	0.01683	8.3545e-005	0.083545

EXPERIMENT 2 (rec. flow 18 mL/min)

time (min)	[BD] g/L	Et*1000
5	0.004256	0.012047
13	0.07721	0.21855
16	0.1117	0.31617
20	0.1357	0.38411
25	0.1645	0.46563
30	0.1863	0.52733
35	0.2003	0.56696
40	0.2125	0.6015
47	0.2245	0.63546
51	0.2293	0.64905
55	0.2343	0.6632
60	0.2359	0.66773
65	0.2377	0.67283
70	0.239	0.67651
75	0.2373	0.67169
81	0.2383	0.67452
86	0.2359	0.66773
90	0.2362	0.66858
95	0.2377	0.67283
100	0.2416	0.68386
119	0.2393	0.67735
133	0.2354	0.66632
146	0.2359	0.66773
159	0.2318	0.65613
200	0.2255	0.63829
231	0.2217	0.62754
278	0.2108	0.59668
305	0.2103	0.59527
700	0.15977	0.45223
1092	0.1248	0.35325
1122	0.1226	0.34703
1143	0.1159	0.32806
1153	0.1153	0.32636
1161	0.1148	0.32495
1191	0.1087	0.30768
1215	0.1099	0.31108
1253	0.1154	0.32665
1279	0.1063	0.30089
1299	0.1026	0.29042
1309	0.1031	0.29183
1331	0.104	0.29438
1340	0.1039	0.2941

1360	0.1027	0.2907
1387	0.1019	0.28843
1427	0.09633	0.27267
1870	0.0682	0.19304
2421	0.0501	0.14181
2964	0.03302	0.093465

EXPERIMENT 3 (rec. flow 45 mL/min)

time (min)	[BD] g/ml	Et*1000
6	0.004136	0.01158
10	0.08031	0.22485
15	0.1203	0.33681
20	0.1406	0.39365
26	0.1675	0.46896
30	0.1902	0.53251
35	0.1986	0.55603
40	0.2231	0.62463
44	0.2312	0.64731
51	0.2344	0.65626
55	0.2365	0.66214
60	0.2343	0.65598
65	0.2379	0.66606
70	0.2418	0.67698
75	0.2423	0.67838
80	0.2433	0.68118
87	0.2463	0.68958
90	0.2466	0.69042
95	0.2441	0.68342
120	0.242	0.67754
135	0.2416	0.67642
145	0.2351	0.65822
160	0.2346	0.65682
205	0.228	0.63835
235	0.2216	0.62043
260	0.2198	0.61539
316	0.2103	0.58879
750	0.1563	0.4376
960	0.1337	0.37433
1111	0.1218	0.34101
1205	0.1131	0.31665
1210	0.1183	0.33121
1215	0.1126	0.31525

1222	0.113	0.31637
1231	0.1119	0.31329
1238	0.1105	0.30937
1246	0.1093	0.30601
1275	0.1082	0.30293
1293	0.1065	0.29817
1313	0.1058	0.29621
1325	0.1043	0.29202
1403	0.09891	0.27692
1692	0.08103	0.22686
2361	0.05089	0.14248
2873	0.03574	0.10006

EXPERIMENT 4 (without recirculation)

time min	[BD] g/L	Et*1000
6	0.00954	0.02734
8	0.0201	0.05763
10	0.04564	0.1308
12	0.05897	0.169
14	0.0835	0.2393
17	0.00436	0.01249
18	0.1327	0.3803
21	0.148	0.4243
23	0.1665	0.4773
26	0.1854	0.5314
29	0.1997	0.5725
32	0.2123	0.6085
36	0.2195	0.6291
40	0.2273	0.6514
44	0.2312	0.6626
48	0.2366	0.6781
51	0.2392	0.6857
54	0.2402	0.6886
56	0.242	0.6936
59	0.2393	0.686
63	0.2402	0.6886
66	0.2405	0.6893
70	0.2419	0.6933
73	0.2411	0.6911
78	0.2422	0.6943
81	0.2427	0.6958
85	0.2415	0.6922
88	0.2417	0.6929

92	0.242	0.6936
97	0.2397	0.6871
100	0.2401	0.6882
103	0.24	0.6878
106	0.2392	0.6857
109	0.2386	0.6839
114	0.2372	0.6799
117	0.237	0.6792
121	0.2378	0.6817
125	0.2377	0.6814
129	0.2382	0.6828
132	0.2382	0.6828
136	0.238	0.6821
170	0.238	0.6821
144	0.237	0.6792
152	0.2359	0.6763
154	0.2346	0.6723
158	0.2338	0.6702
163	0.2349	0.6734
166	0.2343	0.6716
171	0.2334	0.6691
175	0.2327	0.6669
186	0.2315	0.6637
189	0.2323	0.6658
203	0.2307	0.6612
209	0.2315	0.6637
215	0.2314	0.6633
230	0.2283	0.6543
243	0.2246	0.6439
263	0.2248	0.6442
279	0.2222	0.637
301	0.2178	0.6244
319	0.2115	0.6064
332	0.2109	0.6046
248	0.208	0.5963
366	0.2048	0.5869
384	0.2037	0.584
961	0.1201	0.3443
968	0.1258	0.3605
972	0.1239	0.3551
978	0.1266	0.363
992	0.1235	0.354
1028	0.1186	0.3399
1041	0.1183	0.3392
1051	0.119	0.341
1077	0.1168	0.3347
1085	0.1165	0.3338
1094	0.1171	0.3356

1128	0.1142	0.3273
1146	0.1147	0.3288
1180	0.1126	0.3226
1242	0.1087	0.3114
1267	0.1059	0.3035
1303	0.1034	0.2963
1325	0.09985	0.2862
1362	0.1007	0.2887
1382	0.1002	0.2873
1427	0.09796	0.2808
1451	0.09633	0.2761
2021	0.07645	0.2192
2034	0.06476	0.1856
2111	0.06375	0.1827
2149	0.06136	0.1759
2192	0.05985	0.1716
2292	0.05557	0.1593
2357	0.05331	0.1528
2408	0.05306	0.1521

EXPERIMENT 5 (without recirculation)

time min	[BD] g/L	Et*1000
4	0.003502	0.01035
5	0.01294	0.03823
6	0.03231	0.09548
7	0.04551	0.1345
8	0.06488	0.1918
9	0.07897	0.2334
10	0.09394	0.2776
11	0.1089	0.3219
13	0.1336	0.3947
15	0.1538	0.4546
16	0.1616	0.4776
15	0.171	0.5055
16	0.1737	0.5133
20	0.1788	0.5286
22.5	0.1851	0.5471
24	0.1894	0.5598
26	0.1986	0.5869

28	0.2055	0.6074
30	0.2119	0.6263
35	0.2203	0.6512
40	0.2217	0.6553
46	0.2259	0.6676
51	0.2329	0.6884
57	0.2351	0.6947
63	0.2366	0.6992
68	0.2374	0.7018
74	0.2368	0.6999
79	0.2382	0.704
84	0.2377	0.7025
89	0.2381	0.7036
95.5	0.2411	0.7126
102	0.24	0.7092
107	0.24	0.7092
114	0.2407	0.7115
120	0.2405	0.7107
128	0.2381	0.7036
135	0.2368	0.6999
145	0.2353	0.6955
172	0.2304	0.681
228.5	0.2217	0.6553
234	0.2206	0.652
261	0.2172	0.6419
278	0.2146	0.6341
301	0.2118	0.626
335.5	0.2065	0.6103
386	0.1998	0.5906
404	0.1978	0.5847
422	0.1941	0.5735
465	0.1884	0.5568
492	0.1855	0.5483
1333	0.1083	0.32
1349	0.1051	0.3107
1379	0.106	0.3133
1429	0.1009	0.2981
1478	0.09746	0.288
1492	0.09834	0.2906
1509	0.09771	0.2888
1578	0.09306	0.275
1626	0.09067	0.268
1722	0.08652	0.2557
1756	0.09243	0.2732
1796	0.07595	0.2245
1923	0.06979	0.2063
1944	0.07243	0.2141
2770	0.04212	0.1245

2816	0.04023	0.1189
2825	0.04098	0.1211

EXPERIMENT 6 (without recirculation neither aeration)

time min	[BD] g/L	Et*1000
426	0.006269	0.01783
451	0.01344	0.03822
516	0.01268	0.03607
529	0.023	0.0654
536	0.0191	0.05431
548	0.01746	0.04966
557	0.02023	0.05753
563	0.02237	0.06361
568	0.02262	0.06433
577	0.02551	0.07255
584	0.02778	0.07899
596	0.03243	0.09223
602	0.0352	0.1001
609	0.03646	0.1037
624	0.044	0.1251
629	0.04727	0.1344
650	0.09356	0.2661
653	0.08865	0.2521
661	0.1161	0.3301
666	0.08802	0.2503
670	0.09079	0.2582
674	0.08777	0.2496
686	0.1064	0.3025
699	0.1137	0.3233
711	0.1152	0.3276
724	0.1242	0.3533
749	0.1343	0.382
773	0.1465	0.4166
789	0.1469	0.4177
801	0.1514	0.4306
809	0.1539	0.4378
815	0.1688	0.48
821	0.157	0.4463
825	0.1606	0.4567

829	0.1586	0.451
833	0.1611	0.4581
839	0.1626	0.4624
850	0.1664	0.4732
873	0.1695	0.4821
887	0.1709	0.486
916	0.1776	0.505
1131	0.1688	0.48
1141	0.1689	0.4803
2266	0.07922	0.2253
2284	0.07595	0.216
2300	0.07369	0.2095
2323	0.0723	0.2056
2366	0.0718	0.2042
2387	0.07042	0.2002
2435	0.07092	0.2017
2516	0.07482	0.2128
2564	0.06576	0.187
2595	0.06438	0.1831
2630	0.06677	0.1899
2824	0.05583	0.1588
2906	0.06312	0.1795
2967	0.0591	0.1681
3055	0.05256	0.1495
3100	0.05608	0.1595
3134	0.06488	0.1845
4305	0.04501	0.128
4313	0.03331	0.09473
4346	0.02979	0.08472
4361	0.03432	0.09759
4402	0.04111	0.1169
4416	0.03885	0.1105
4446	0.03017	0.08579