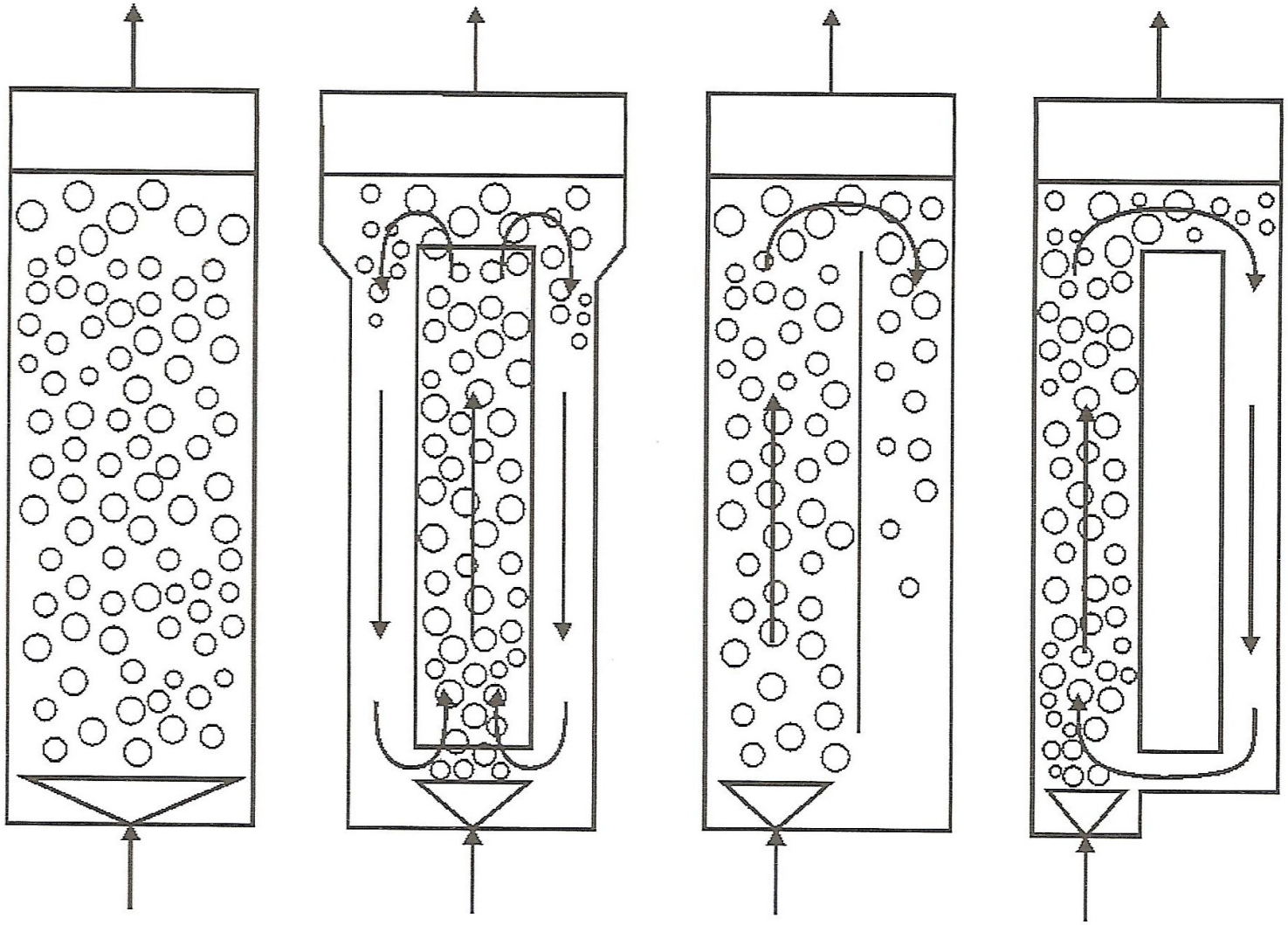
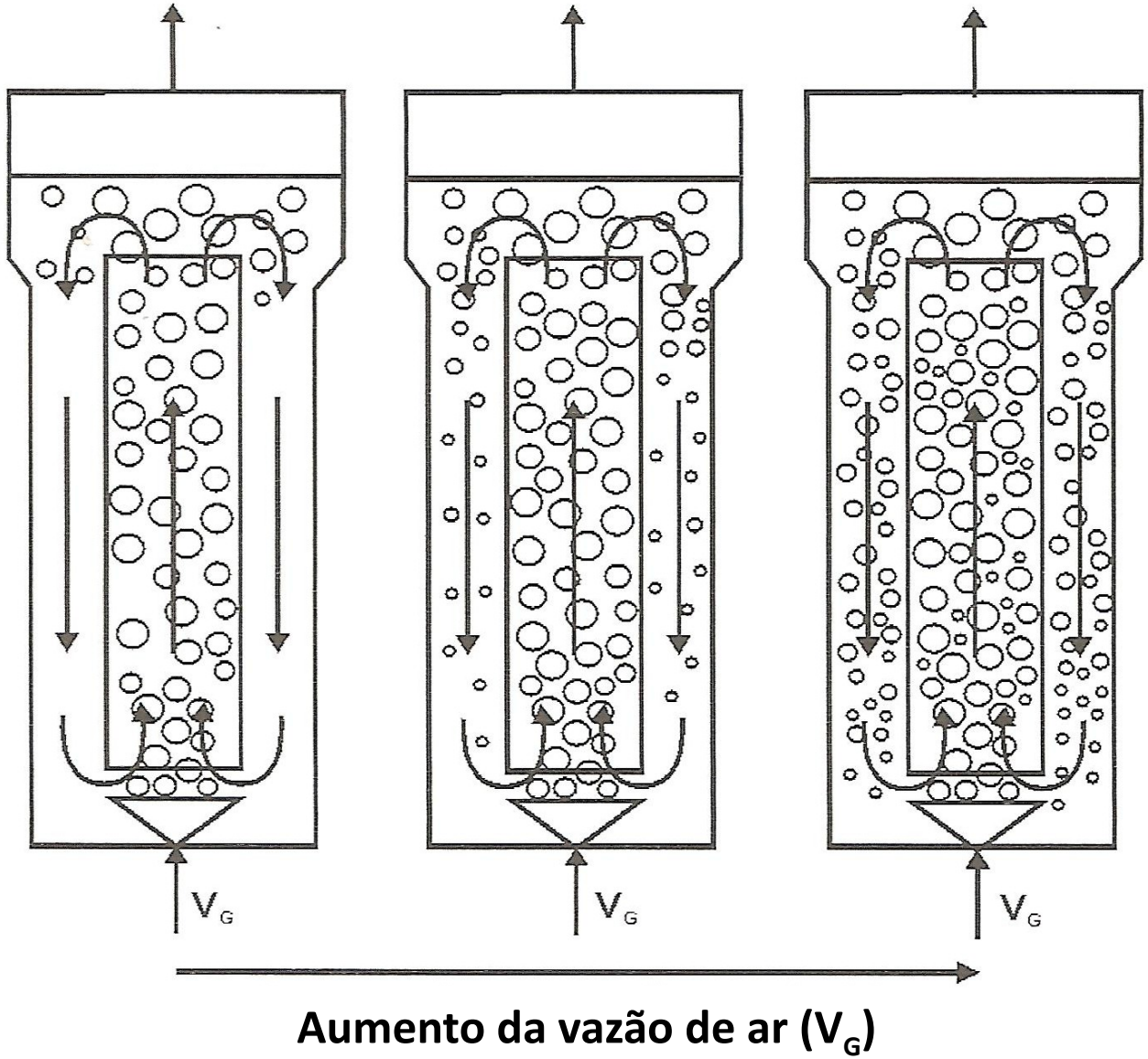
Universidade de São Paulo
Escola de Engenharia de Lorena

Transferência de potência e de oxigênio em sistemas agitados e aerados

Algumas considerações sobre reatores coluna de bolhas e “air lift”



Diferentes concepções de reator “air lift” (esquerda para direita): coluna de bolha normal; “draft tube”; “air lift” com parede de separação; “air lift” com “downcomer” externo.



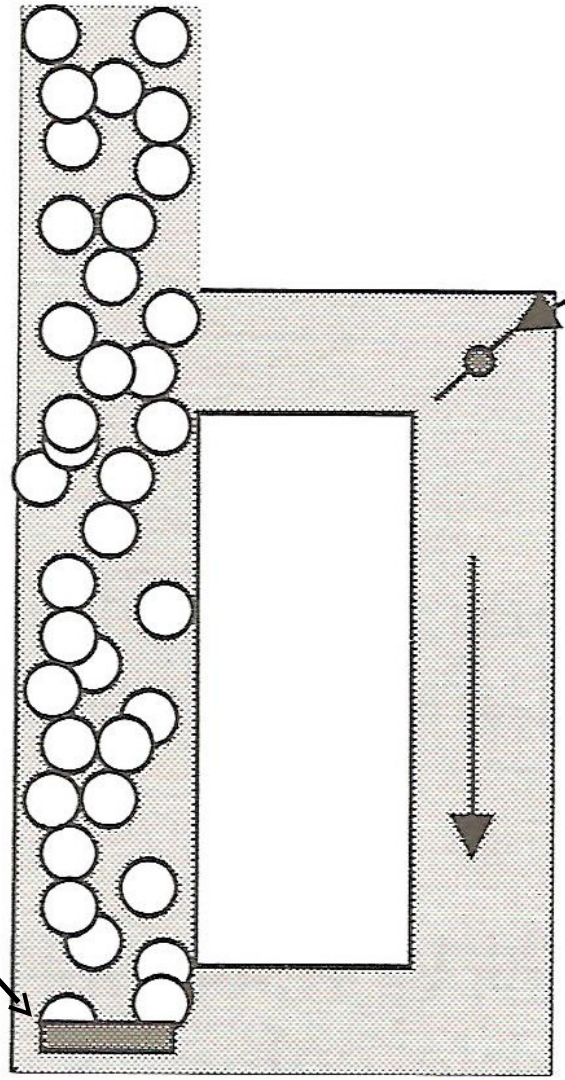
Diferentes condições de circulação de meio e auto-regulação em reator “draft tube”.

Comparação entre coluna de bolha de “air lift”

Verlaan, P. Modelling and characterization of na airlift-loop bioreactor. PhD thesis, Wageningen. 1987.

válvula

dispersor



Esquema de um reator “air lift” com retorno externo.

Para uma mesma vazão de ar, o reator “air lift” apresenta:

- menor “hold up”
- maior tempo de mistura
- menor eficiência energética para transferência de massa

Vantagem: permite empregar maiores vazões de ar que no coluna de bolhas, uma vez que a probabilidade de transbordamento é menor

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Regular article

Hydrodynamics of Newtonian and non-Newtonian liquids in internal-loop airlift reactors

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Scale-up

ABSTRACT

Airlift reactors have numerous applications in processing industries, and thus the hydrodynamic behaviors using fluids that have distinct rheological characteristics need to be better understood for the successful design and operation of these devices. In this sense, the novelty of this study is to evaluate the influence of the energy losses in each reactor region on the interstitial liquid velocity (V_{LR}), and to provide correlations for prediction of gas hold-ups (ε_R and ε_D) and V_{LR} for a wide range of physical and rheological properties of the liquid, using different models (draft-tube—DTA, split-cylinder—SCA) and scales (5 and 10 L) of airlift reactors. Gas hold-ups were determined by a manometric method, and V_{LR} was calculated by circulation time of spheres with the same liquid density. Energy losses were calculated using a semi-theoretical method. The total energy dissipated in the riser and downcomer was greater for DTA reactors, whereas in the bottom was greater for SCA reactors. Non-Newtonian fluids showed higher V_{LR} in SCA reactors, while the opposite was observed for Newtonian fluids. Excellent fits to the experimental data were achieved using simple correlations to predict ε_R and ε_D, as well as dimensionless correlations that incorporated the desired parameter (V_{LR}).

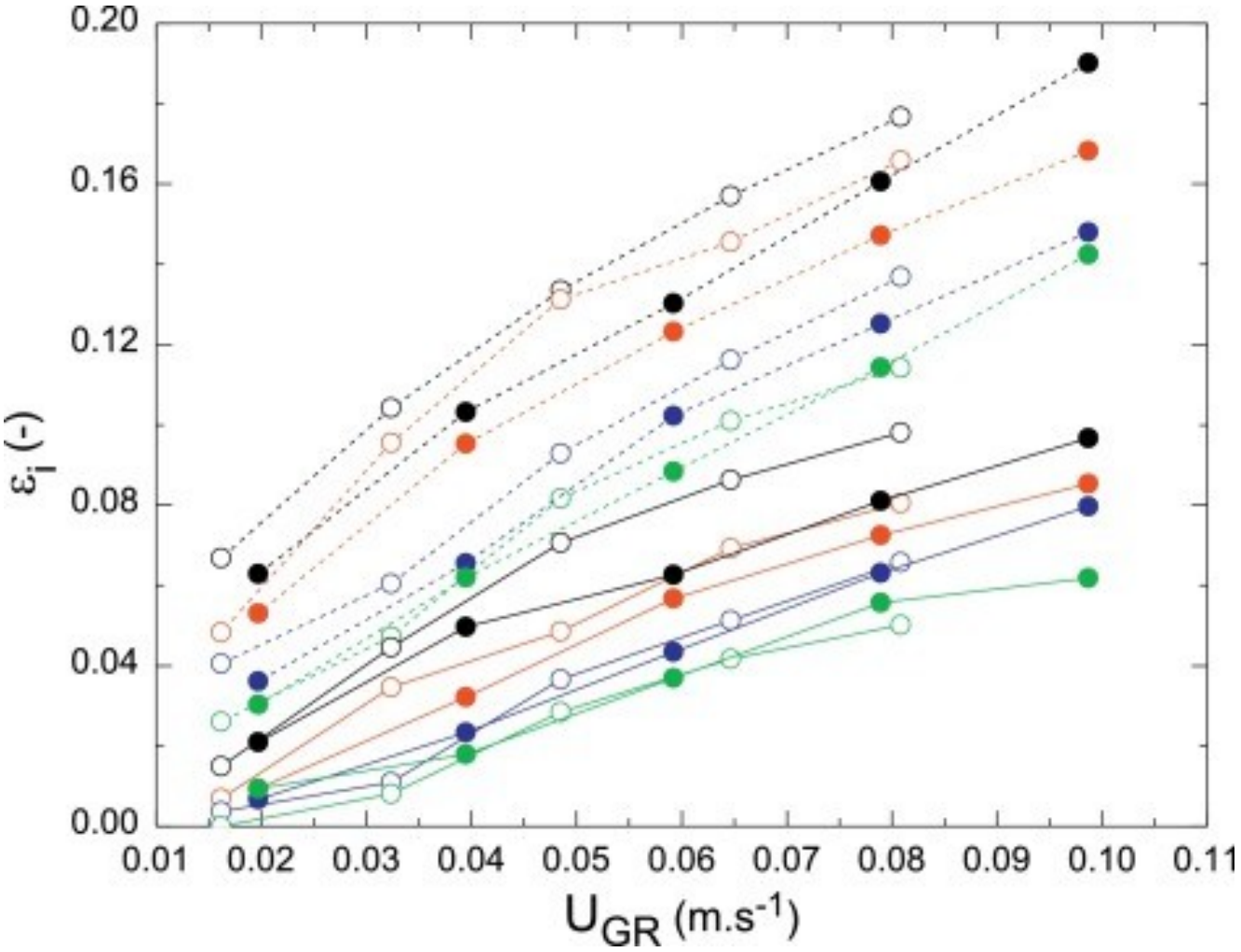
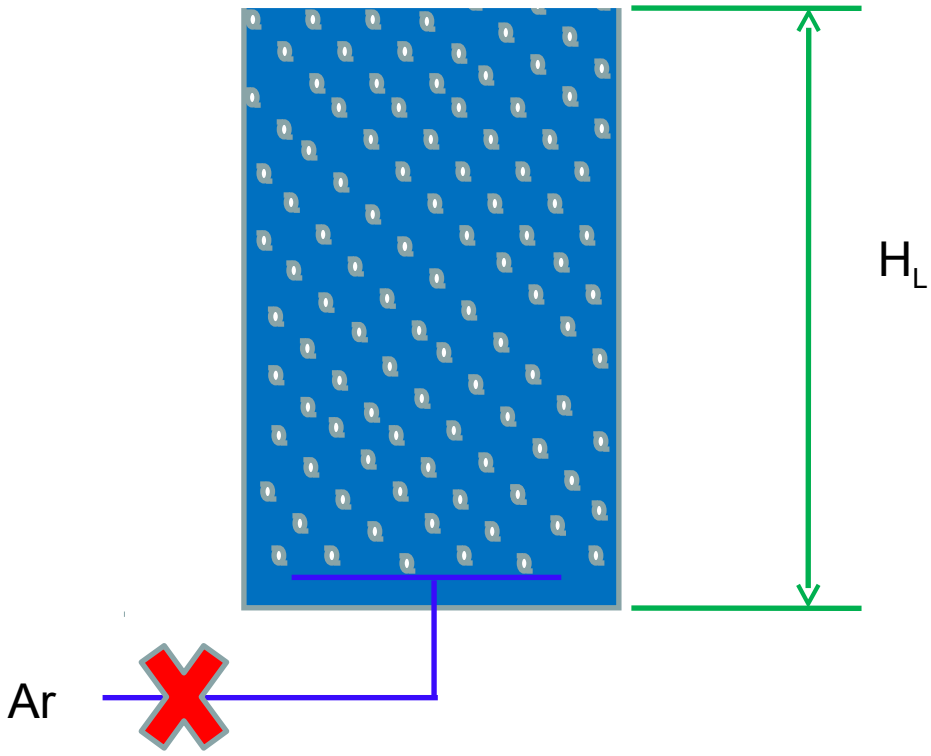


Fig. 2. Typical behavior of the gas hold-up in the riser and downcomer (ε_i) as a function of superficial gas velocity in the riser (U_{GR}), found for Newtonian and non-Newtonian fluids in DTA and SCA reactors. The plotted data refers to the CMC solutions in SCA reactor. Legend: (**O**) CMC15-5L, (O) CMC25-5L, (O) CMC35-5L, (O) CMC45-5L, (●) CMC15-10L, (●) CMC25-10L, (●) CMC35-10L, (●) CMC45-10L; (---) ε_R , (‾‾) ε_D.


H_L
Ar

Alguns casos

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Research Article

Two-compartment processing as a tool to boost recombinant protein production

Pichia pastoris is used extensively as a production platform for many recombinant proteins. The dissolved oxygen (DO) is one of the most important factors influencing protein production. The influence of the DO on productivity has not been studied independent from the feed rate. In this work, various DO levels were investigated independent from the feed rate. The model system was recombinant *P. pastoris* under the control of methanol-induced alcohol oxidase promoter, which expressed HRP as the target protein. No significant effect was observed in terms of titer and specific productivity, which is a confirmation of the fact that the DO in a one-compartment system cannot boost productivity for the model system under study. Hence, a two-compartment system (a single reactor coupled with a plug flow reactor) was designed and implemented in order to apply oxygen-related stress in the plug flow reactor and allow the cells to be recovered in the main reactor. Doing so, more than two-fold increase in the titer and productivity and three-fold increase in protein-specific activity were achieved. Hence, partial application of oxygen-related stress in the two-compartment system was proposed as a process technology to enhance protein production.

Keywords: Dissolved oxygen / Horseradish peroxidase / Oxygen-related stress / *Pichia pastoris* / Two-compartment system

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Introdução

- *Pichia pastoris* é uma levedura muito usada para produção de proteínas recombinantes
- Um dos fatores mais importantes que influenciam a produção é a concentração de oxigênio dissolvido (OD)
- O processo é realizado em sistema descontínuo alimentado e envolve 4 fases: descontínua, descontínua alimentada, adaptação e indução
- Para a melhoria do processo, propôs-se estabelecer choques de concentração de OD em uma situação de mesma velocidade de consumo de substrato
- Para comparação realizou-se 4 ensaios no sistema convencional (biorreator de 3 L)

Material e métodos

- Microrganismo: *Pichia pastoris* contendo o plasmídeo para a síntese de HRP (Horse Radish Peroxidase)
- Substrato: glicerol
- Fases do processo:

Fase descontínua: até 20 g/L de células

Fase descontínua alimentada: até 63 g/L de células

Fase de adaptação: ao metanol

Fase de indução: síntese da proteína “ppd”

- Controle do nível de OD: só variação da agitação
- T = 28 ^{o}C; pH = 5,0; Aeração = 2 vvm

Ensaios realizados em reator convencional

Table 1. One-compartment experiments: experiments with different DO levels done in a single reactor.

Run number	Designation	DO level in induction phase
1	R1	60%
2	R2	25%
3	R3	3%
4	R4	Uncontrolled but >25%

Configuração proposta (dois compartimentos)

- *Associação do biorreator convencional com um reator "plug flow" (PFR)*
- Inclusão de um dispositivo "armadilha" entre os dois reatores para eliminar total ou parcialmente as bolhas de ar do meio

  Isso possibilita estabelecer diferentes níveis de OD no reator *PFR*
- No reator convencional (STR) manteve-se o mesmo nível de OD para todos os ensaios

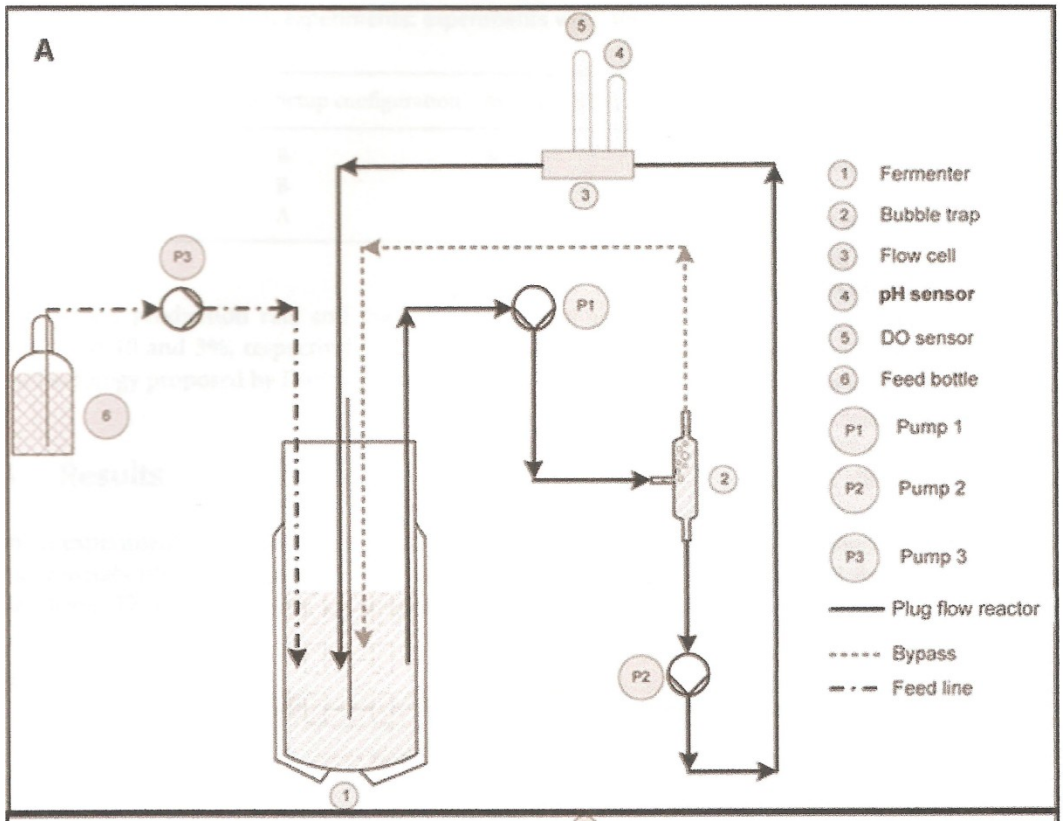
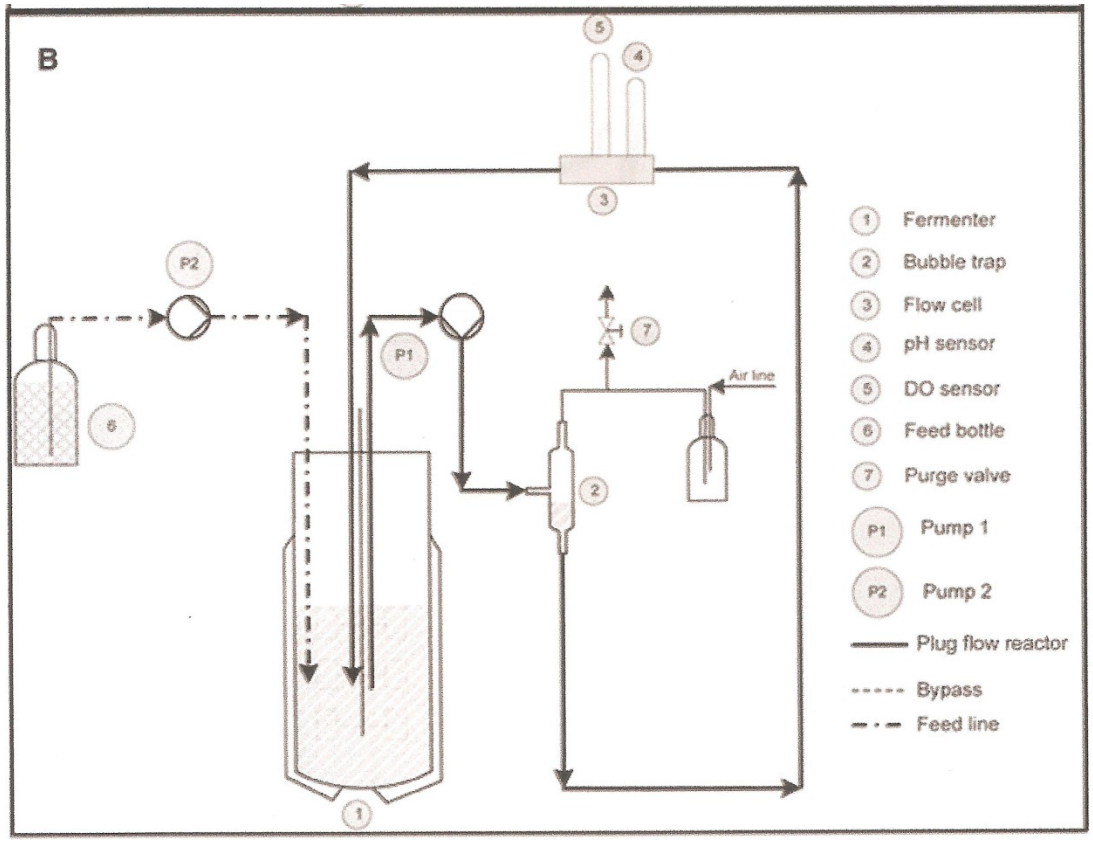


Figure 1. Schematic view of the two-compartment system: a single fermenter coupled with a PFR. The volume of the PFR was 90 mL. The circulation flow was adjusted to 27.3 L/min. The operating condition in the main reactor was 28°C, 2 vvm, pH 5. (A) Bubble elimination in the PFR is done by means of a bubble trap (2). Bubble containing stream flows back to the fermenter via a bypass line (gray dashed line). (B) Bubble elimination in PFR is done by means of balancing the overhead pressure of bubble trap via the purge valve (7). Bubbles are separated from the stream and vented out through the purge value.



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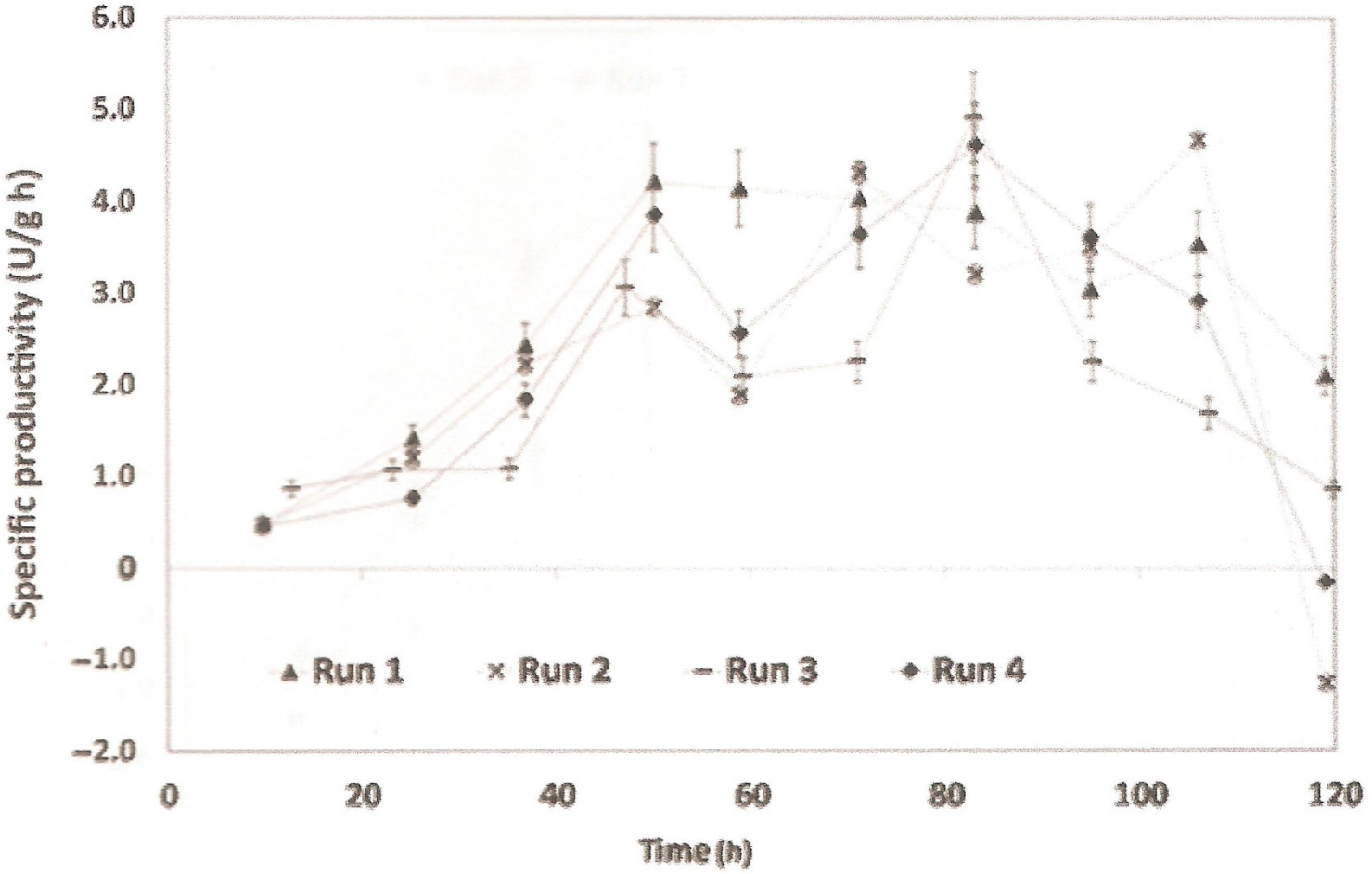
Ensaios realizados com a estratégia de dois compartimentos

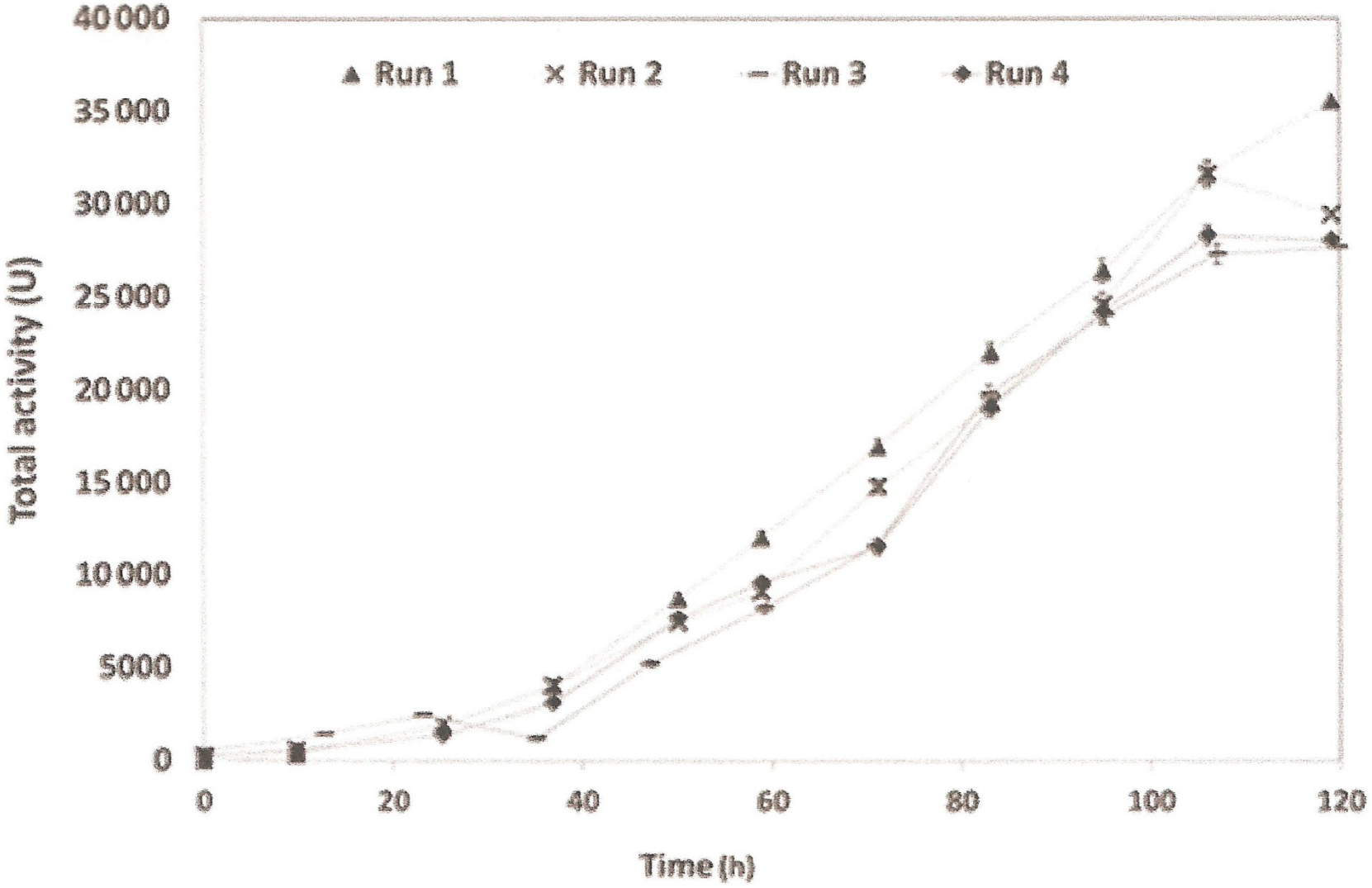
Table 2. Two-compartment experiments: experiments with different DO levels in the PFR.

Run number	Designation	Setup configuration	Average DO in PFR
5	R5	B	30%
6	R6	B	10%
7	R7	A	0%

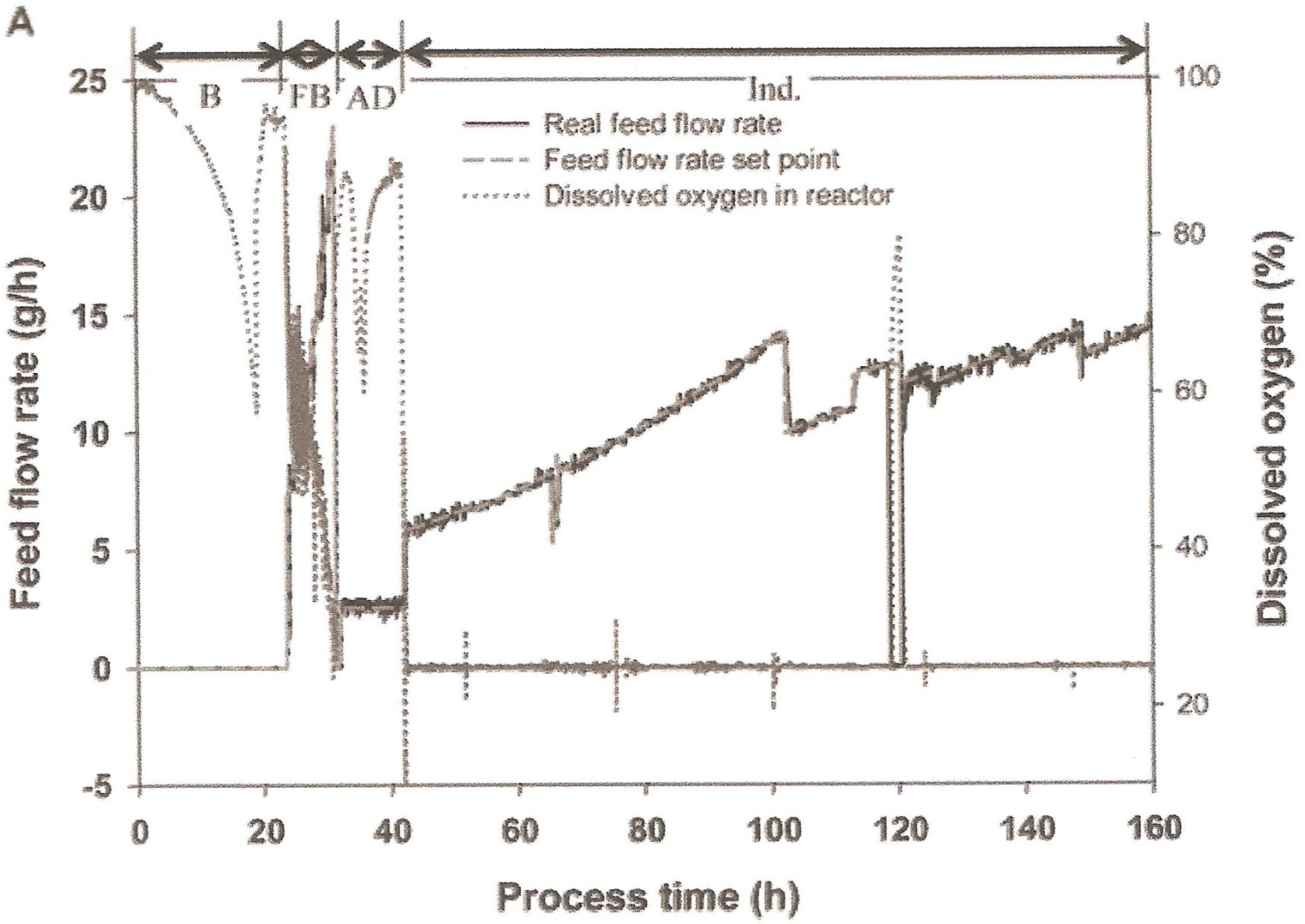
Resultados

Ensaios no biorreator convencional

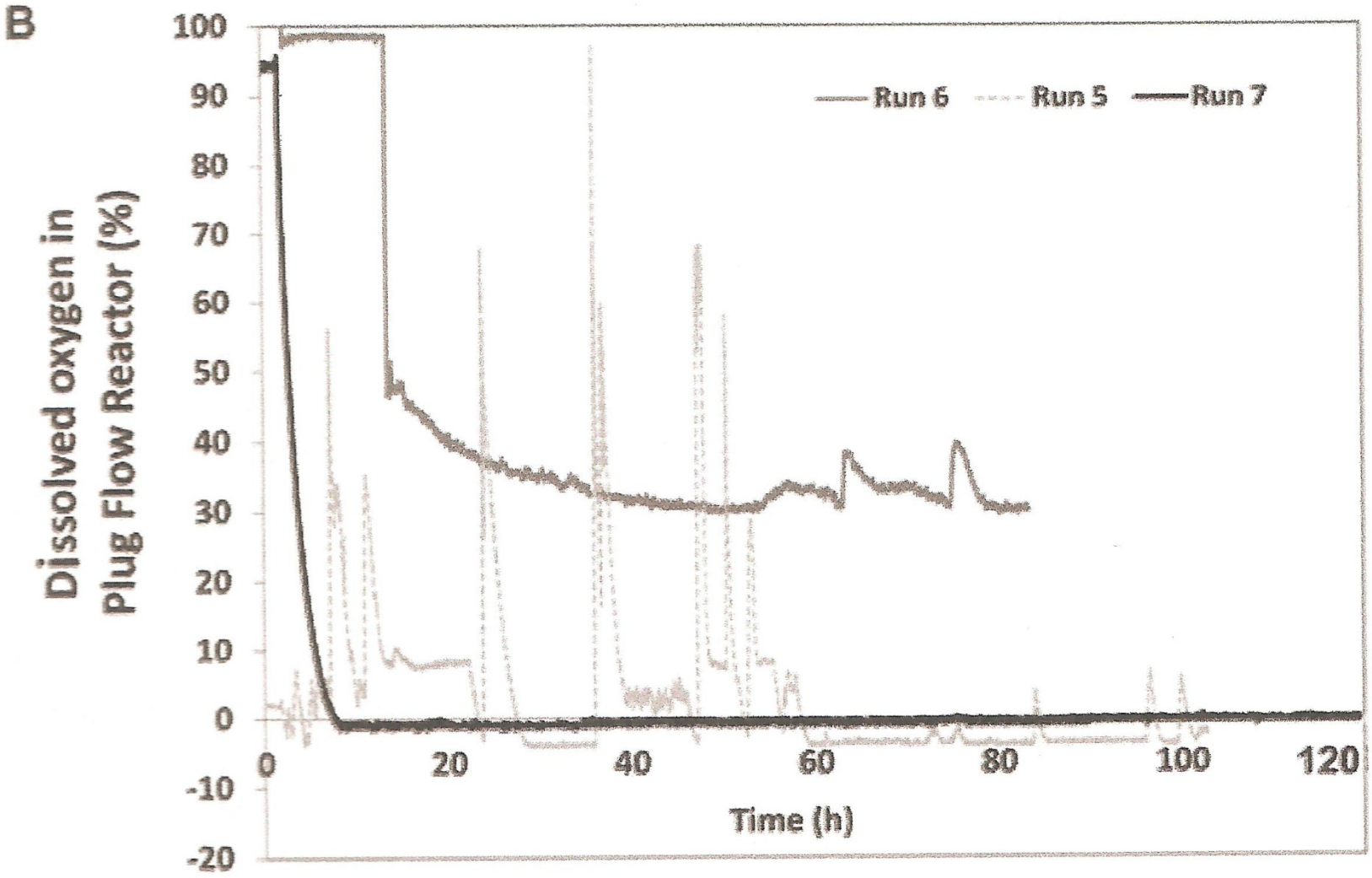



40 000
35 000
30 000
25 000
20 000
15 000
10 000
5000
0
Total activity (U)
Run 1
Run 2
Run 3
Run 4
0
20
40
60
80
100
120
Time (h)

Ensaios no sistema de dois compartimentos

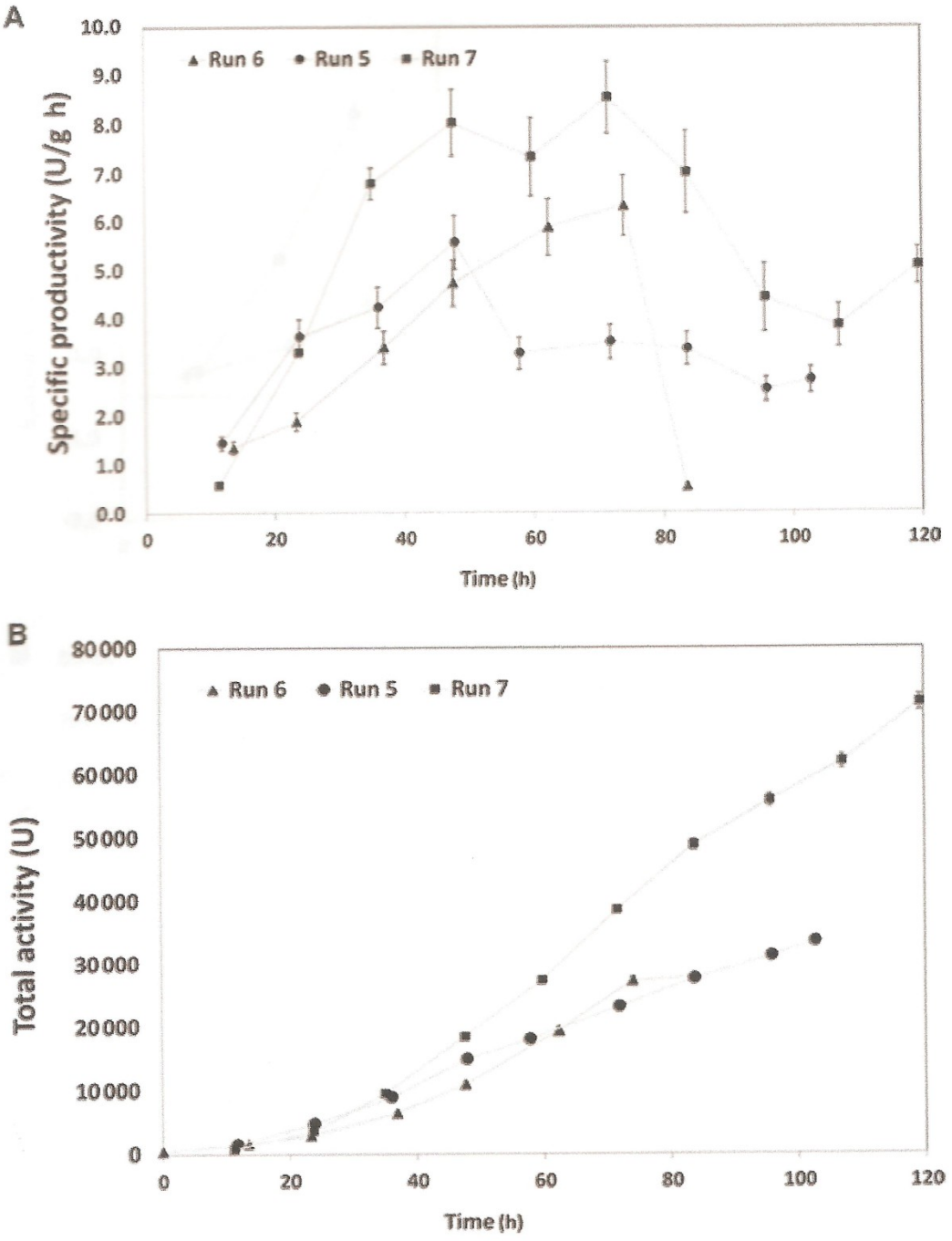


Exemplo do monitoramento do processo *no biorreator* (ensaio 7).

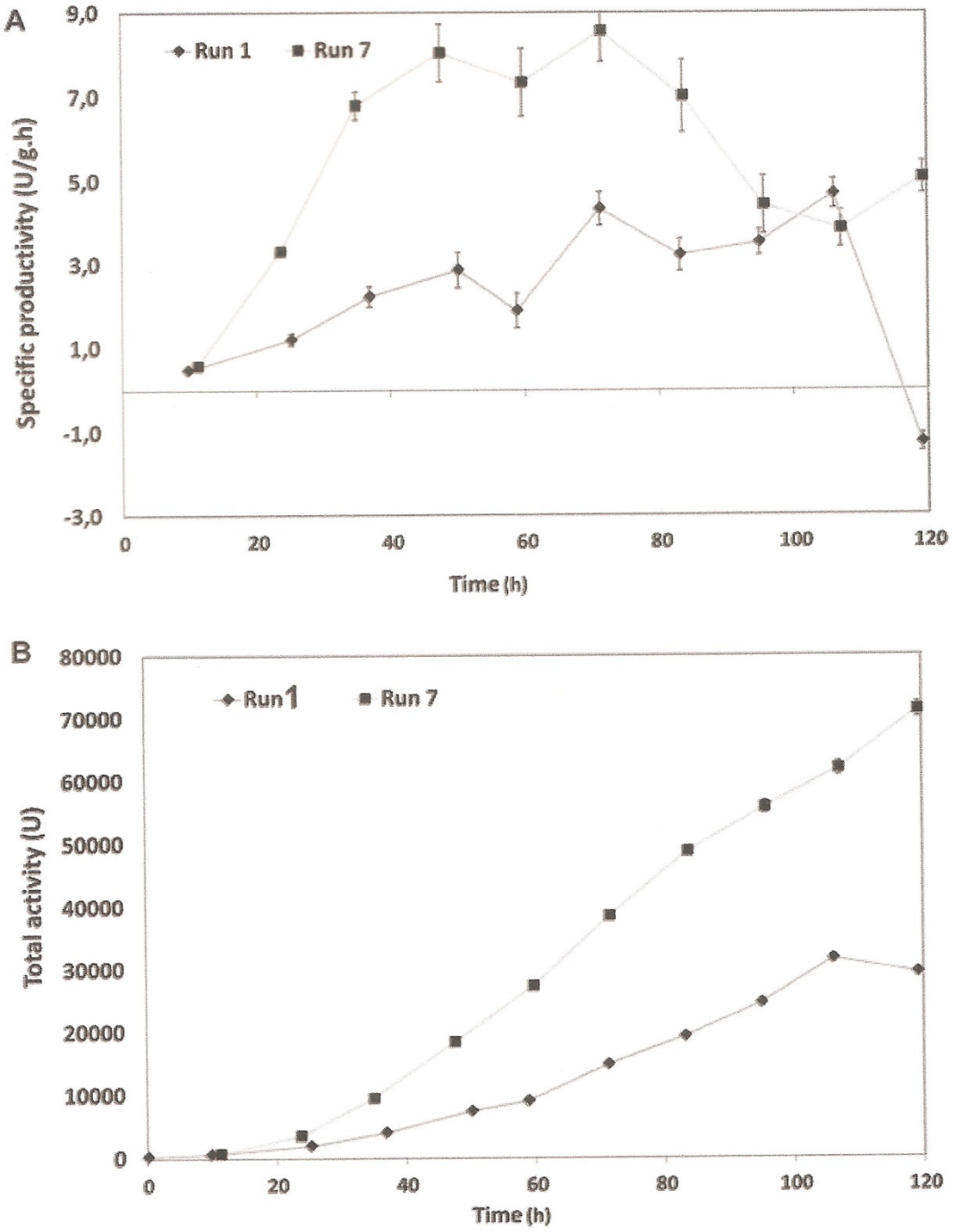


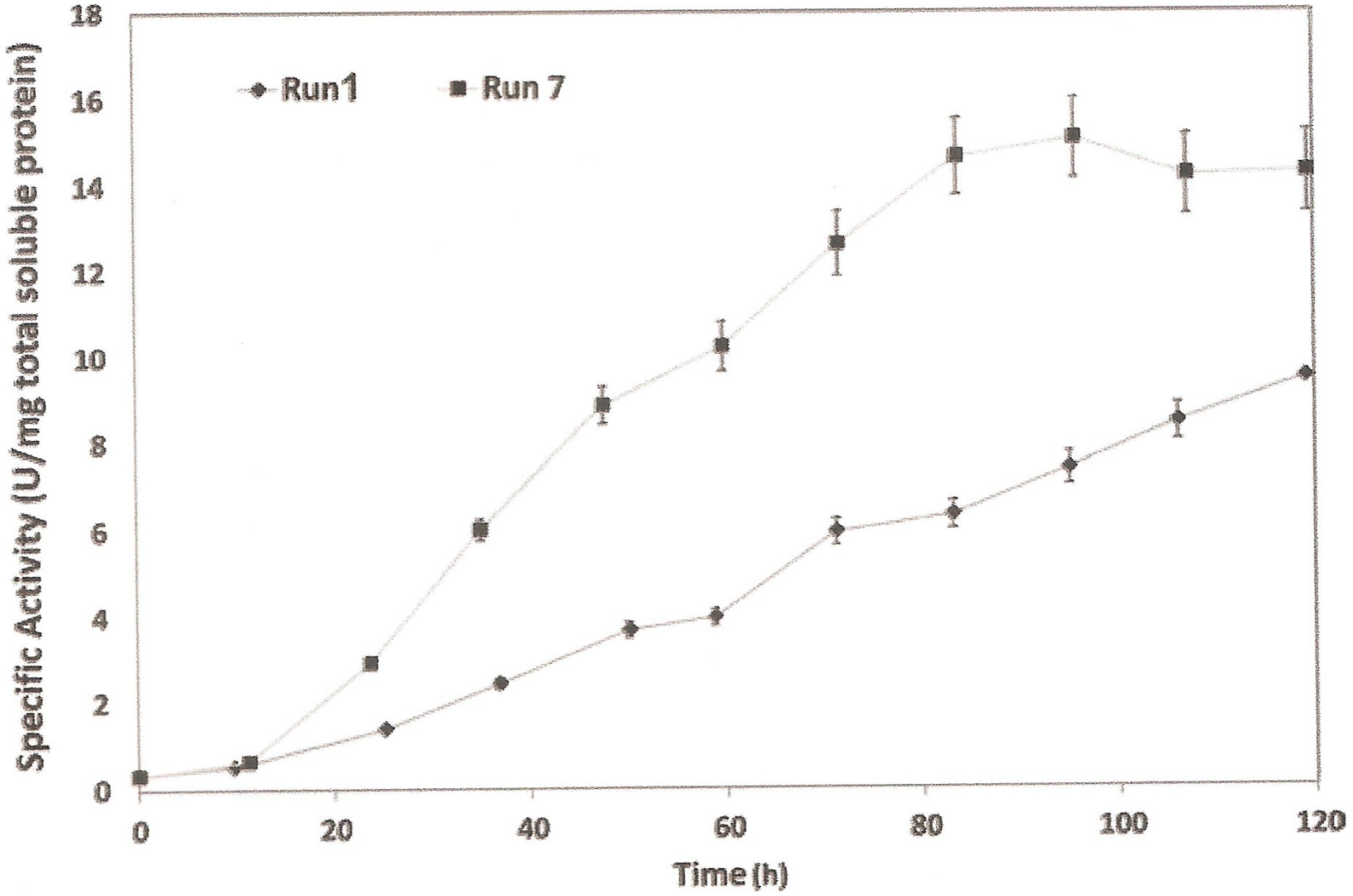
Monitoramento do nível de OD *no reator PFR* (ensaios 5, 6 e 7).

Produtividade específica e atividade total de *HRP* dos ensaios **5**, **6** e **7**, com o sistema de dois compartimentos.



Produtividade específica e atividade total de *HRP* dos ensaios **1** e **7**, com o sistema convencional e de dois compartimentos, respectivamente.





Atividade específica de *HRP* dos ensaios **1** e **7**, com o sistema convencional e de dois compartimentos, respectivamente.

Conclusão

- Diferentes níveis de OD na fase de indução não melhoram a produção de HRP
- A estratégia do sistema de dois compartimentos permite aumentar a produção e a atividade de HRP, constituindo, assim, uma ferramenta potencial para aumentar a produção e a atividade de outras proteínas
- A estratégia permite reduzir a potência por volume (P/V), pela redução da velocidade de agitação
- A limitação completa de oxigênio no reator PFR é necessária para aumentar a produção de HRP
- A produção pode ser ainda melhorada otimizando-se o tempo de residência no PFR

Gas Transfer and Liquid Dispersion Inside a Deep Airlift Reactor

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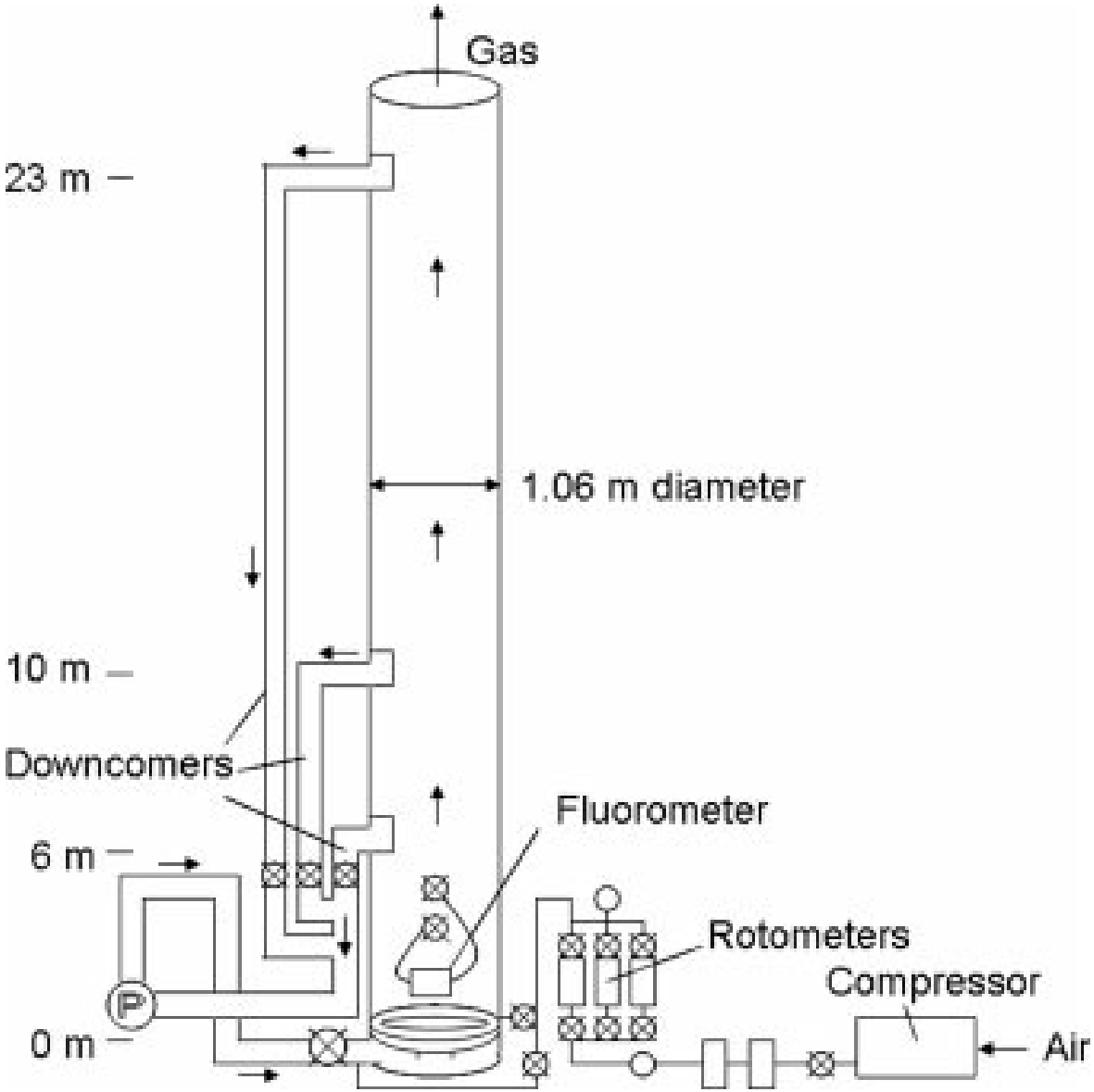
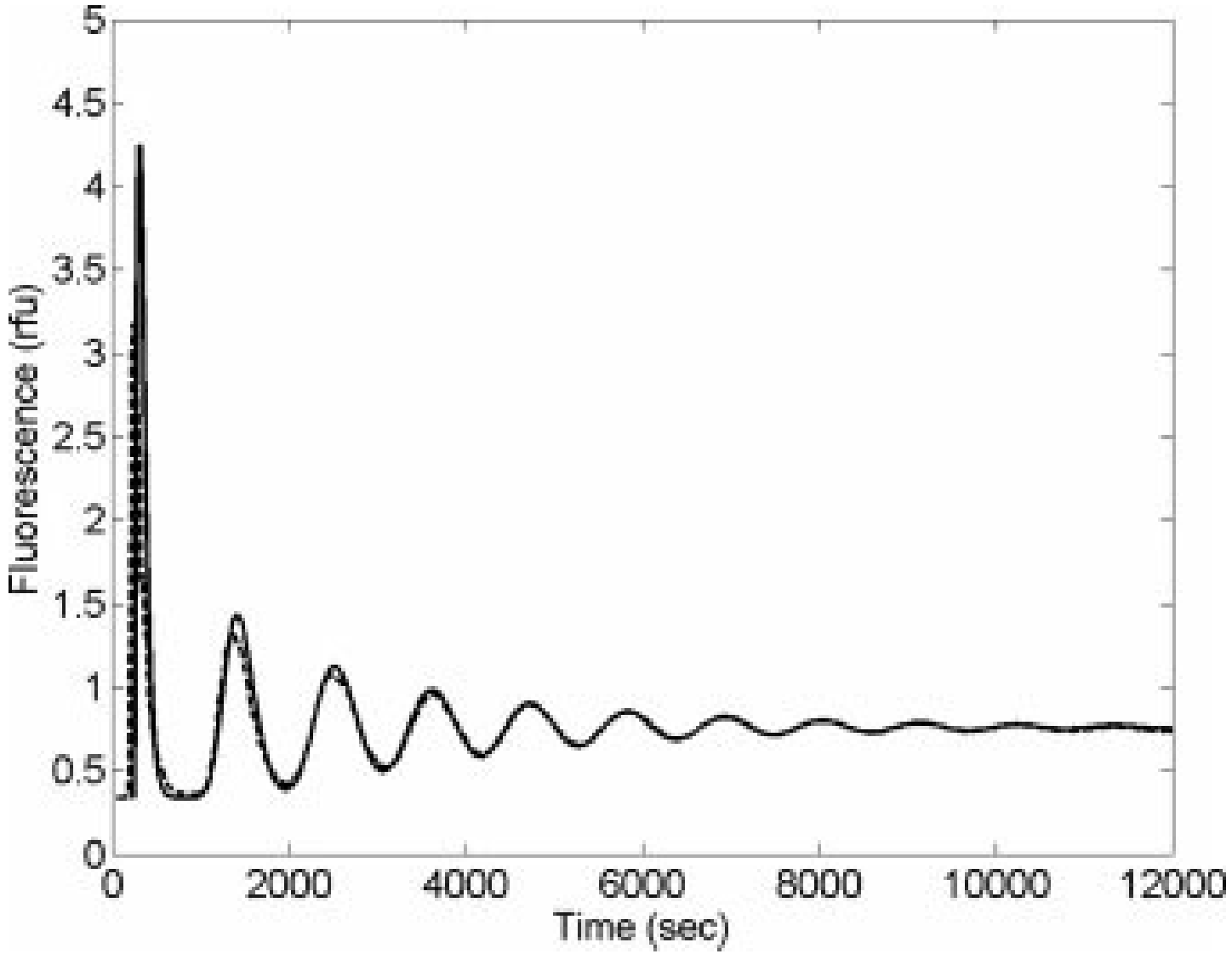
DOI 10.1002/aic.11449
Published online February 21, 2008 in Wiley InterScience (www.interscience.wiley.com).

Gas transfer experiments in bubbly flow are conducted inside a deep bubble column/airlift reactor containing air and water with a maximum aerated water height of 23.4 m and diameter of 1.06 m. The effects of geometry and operating conditions on mixing and gas transfer are determined. Fluorescence measurements are used to estimate dispersion coefficients for several operating conditions, while bubble-water gas transfer measurements are made using dissolved oxygen (DO) probes. A two-phase convection-dispersion model is fit to the DO measurements using the liquid film coefficient (k_L) as a fitting parameter. Sparger differences had a substantial effect upon k_L, and the gas transfer coefficient for the airlift reactor was four times that of the bubble column. Results are characterized using Sherwood, Reynolds, and Bond numbers. A low Reynolds number exponent was found, indicating that k_L in a deep column tends toward a constant and is not highly dependent upon air discharge. *AIChE J*, 54: 850–861, 2008
Keywords: bubble column, airlift reactor, gas–liquid systems, gas–liquid mass transfer, dispersion, aeration

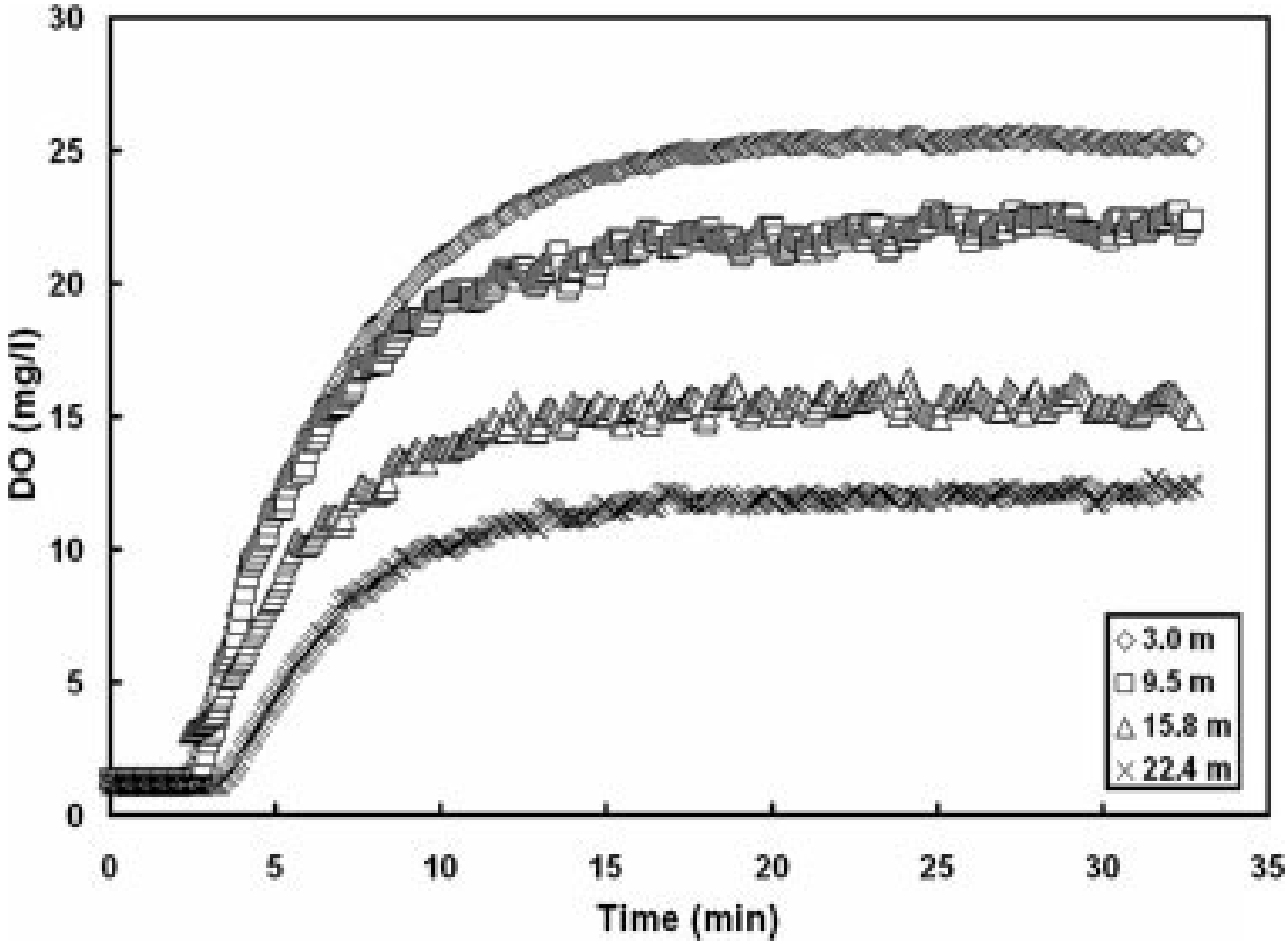


Esquema do reator para ensaios de transferência gasosa.



Exemplo de perfil de concentração de corante (pontos) e curva obtida por modelo (linha sólida) numa condição sem aeração, empregando a placa perfurada e altura de 22,4 m.

Fotos do aspersor (menores orifícios e em maior número) (a) e do difusor de bolhas (maiores orifícios e em menor número) (b).



Perfis de concentração de OD medida a várias alturas no reator “air lift” de 23,4 m, empregando uma placa perfurada como aspersor de ar, com μ_g = 0,01 m/s.