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Effect of fluiddynamic conditions on growth rate and biodesulfurization capacity of *Rhodococcus erythropolis* IGTS8

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ABSTRACT

The growth rate and desulfurization capacity accumulated by the cells during the growth of *Rhodococcus erythropolis* IGTS8 under different fluid dynamic conditions in a stirred and sparged tank bioreactor have been studied. Hydrodynamic conditions were changed using different stirrer speeds and gas flow rates. It was observed that the growth was strongly dependent on the stirrer speed employed. Oxygen transfer limitation was observed at low stirrer speeds (from 100 to 250 rpm). In contrast, at higher stirrer speeds, cell damage was caused by hydrodynamic stress in the turbulent bulk of the broth, yielding again a decrease in growth for stirrer speeds higher than 450 rpm. Moreover, increasing the agitation from 100 to 450 rpm has a positive influence on the development of the desulfurization capacity of the cells during growth, yet this capacity shows a dramatic decrease for higher stirrer speeds. Nevertheless, the change of the air flow rate hardly has any influence on the growth rate and no hydrodynamic stress effect has been detected between 1 and 10 L min^{-1}. A regime analysis of the characteristic times for oxygen mass transfer, oxygen uptake and mixing under different agitation conditions has been made. It was found that the minimum stirrer speed necessary for a satisfactory performance of the bioreactor from the point of view of the cells oxygen demand was 350 rpm, while at stirrer speeds over 450 rpm, growth rate and desulfurization capacity are both negatively affected.

1. Introduction

In aerobic industrial bioprocesses, usually carried out in stirred tank bioreactors (STBR), power dissipation per unit of volume is a critical aspect in the scale-up and operation, determining the productivity of the bioprocess due to their relevant influence on the hydrodynamic conditions of the bioreactor. The increase of the power dissipation per volume unit usually is, caused by an increase of the agitation rate, beneficial in most bioprocesses due to the improvement of mass transfer rates and mixing; however, excessive agitation causes a concomitant increase in hydrodynamic forces that may become a negative factor due to the interaction of turbulence with living cells. Sparging of gas may produce an increase in turbulence. Bubble disengagement at the liquid surface produces dramatic changes in local velocity driven by surface tension, and it may also have a negative influence on the performance of suspended cells.

The effect of turbulence on suspended cells is one of the most complex problems in the scale-up of cell cultures because shear effects of flow turbulence inside the bioreactor may have an important impact. For example, the following can be changed as a result of hydrodynamic stress and cell damage: growth and production rates [1–4], product distribution by selection of metabolic routes due to the transport rate of nutrients affecting the enzyme activities [5], oxygen consumption rate and oxygen dissolved concentration [6,7]. Several approaches have been followed to evaluate the shear sensitivity of cell suspension cultures and to determine the fluid dynamic restrictions that such sensitivity imposes, depending on the microorganism employed as biocatalyst. These restrictions can determine the bioreactor operating conditions, the scale-up criteria and even deciding the type of bioreactor to be used. In some studies, the problem has been envisaged in terms of threshold values of either shear rate, shear stress or stirrer speed, above which the growth rate and cell viability [3,8,9], product yield [10,11], or some other critical culture parameter are significantly affected [12,13].

Biodesulfurization (BDS) is a promising process and a complementary technology for desulfurization of some petrochemical fractions. BDS biocatalysts are microorganisms selected for their ability to specifically degrade some sulfur-containing organic compounds. A key organism in this context is *Rhodococcus erythropolis* IGTS8, the first strain discovered to be able to extract sulfur from a variety of sulfur-containing organic compounds. This gram

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Nomenclature

C^*	Concentration in equilibrium (mol m^{-3})
C_j	Concentration of compound j (mol j L^{-1})
C_{O_2}	Oxygen dissolved concentration (% or mol m^{-3})
D	Stirrer diameter (m)
DBT	Dibenzothiophene
F	Fischer statistical parameter
HBP	2-Hydroxybiphenyl
k_La	Volumetric oxygen mass transfer coefficient (s^{-1})
m_{O_2}	Dissolved oxygen consumption coefficient (mol O_2 kg X^{-1} s^{-1})
N	Stirrer speed (rpm or rps)
N_P	Stirrer power number
OTR	Oxygen transfer rate (mol O_2 m^{-3} s^{-1})
OUR	Oxygen uptake rate (mol O_2 m^{-3} s^{-1})
P	Power input to the bioreactor (W)
P_0	Power input in unaerated systems (W)
Q	Volume flow rate of gas (L min^{-1})
q_{O_2}	Specific oxygen uptake rate (mol O_2 kg X^{-1} s^{-1})
Re	Impeller Reynolds number
SSR	Sum of square residuals
T	Vessel diameter (m)
t	time (s or h)
V	Volume (m^3)
Y_{O_X}	Macroscopic specific yield of oxygen (mol $O_2{}^{-1}$ kg X)

Greek letters

ϵ	Total energy dissipation rate per unit mass (W kg^{-1})
η	Effectiveness factor for growth (–)
μ	Maximum specific growth rate (h^{-1})
μ_0	Maximum or formal specific growth rate (h^{-1})
μ_a	Apparent viscosity (Pa s)
ρ	Density (kg m^{-3})

Subscripts

G	Relative to gas phase
L	Relative to liquid phase
max	Referred to maximum value
MIX	Relative to mixing
O_2	Referred to oxygen
OTR	Relative to mass transfer
OUR	Relative to oxygen uptake rate
X	Referred to biomass

Superscripts

t	Referred to time of 120 min
0	Referred to initial value

positive bacterium is able to convert dibenzothiophene (DBT) to 2-hydroxybiphenyl (HBP) and sulphate through the 4S pathway, which is catalyzed by four enzymes (D*szA, DszB, DszC and DszD*) [14]. The genes involved in the biodesulfurization steps have been characterized, being called *dszA*, *dszB* and *dszC*. They have been located in a single operon on a 150 kb circular plasmid, and on a 100 kb linear plasmid in related strains [15,16]. This bacterium is unable to use DBT as a source of carbon and HBP is not further metabolized [17].

In previous works [18,19], the growth and the biodesulfurization capacity of *R. erythropolis* IGTS8 culture were studied. The medium composition and the operating conditions (temperature, pH and dissolved oxygen concentration) for obtaining a high rate of both growth and biodesulfurization capacity were established. Kinetic models for both growth and biodesulfurization were proposed. In other work [20], it was established that the growth rate and the percentage of biodesulfurization are affected by the volumetric mass transfer coefficient, which is strongly affected by the stirrer speed in a STBR.

Those previous results suggest that the dissolved oxygen concentration is a key factor in the flow of source carbon for cell growth and biodesulfurization capacity of *R. erythropolis* and that both may be modified by power input. This not only affects the growth rate, but also the enzymes developed by the cells, which are afterwards used in biodesulfurization being measured by resting cell assays. It is likely that an optimal agitation level exists that yields the optimal growth rate, or else a biomass concentration with the optimal enzymatic activities. Nevertheless, these aspects concerning hydrodynamic conditions remain ambiguous. For cell suspension cultures, the independent effect of agitation is difficult to quantify as it is coupled with various other phenomena (mixing and oxygen transfer rates and hydrodynamic stress, mainly). In many cases, microbial growth rate has been claimed to be increased by improving the oxygen transfer rate; therefore, a positive effect of agitation is asserted, being also able to compensate the possible cell damage by shear stress. To better understand the influence of hydrodynamic conditions in the bioreactor on the biocatalyst production of *R. erythropolis* strain IGTS8, it is important to separate the positive influence of the transport rate from the possible damage due to shear effects. Thus, for an adequate description of the system, it is necessary to know the dependence of the oxygen transfer coefficient on fluid dynamics and the evaluation of the associated physical and biological parameters.

The aim of this work is to study the effect of hydrodynamic conditions on bacterial cultures of *R. erythropolis* IGTS8 under different operating conditions in a stirred tank bioreactor. Hydrodynamic conditions were changed varying agitation and aeration. Growth runs have been performed under different stirrer speeds and gas flow rates. A kinetic model able to fit the growth curves has been applied and the values of the kinetic parameters have been related to the different conditions used. The effect on the desulfurization capacity accumulated by the cells during growth was evaluated by means of resting cell assays.

2. Materials and methods

2.1. Microorganism and culture medium

The microorganism used has been *R. erythropolis* IGTS8, supplied by the Centro de Investigaciones Biologicas (CIB, Madrid, Spain).

The strain was maintained at 4 °C on LB-Agar plates and transferred every 14 days. The inoculum build-up was made in two stages, under the conditions described elsewhere [19]. Pre-inoculum was prepared in LB medium and 50 mL into a 250 mL. Erlenmeyer flasks were inoculated with a loop of cells from the LB-Agar plate and incubated in a Gallenkamp (model INR-200) orbital shaker at 210 rpm, 30 °C, during 24 h. Afterwards, this culture was used to inoculate a 50 mL in a 250 mL Erlenmeyer flask, fixing initial concentration at 0.1 g L^{-1}. Cells culture was carried out under the same conditions, during 12 h.

The culture medium composition for growth was a standard medium (BSM) of the following composition: $NaH_2PO_4{\cdot}H_2O$, 4 g L^{-1}; $K_2HPO_4{\cdot}3H_2O$, 4 g L^{-1}; $MgCl_2{\cdot}6H_2O$, 0.0245 g L^{-1}; $CaCl_2{\cdot}2H_2O$, 0.001 g L^{-1}; $FeCl_3{\cdot}6H_2O$, 0.001 g L^{-1}; glycerol 2% (w/w); glucose, 20 g L^{-1}, NH_4Cl, 2 g L^{-1} and dimethylsulfoxide, 0.084 g L^{-1}.

2.2. Cell suspension cultures

Bioreactor experiments were completed in a 2 L working volume stirred tank equipped with pH, dissolved oxygen concentration,