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Decoupling of oxygen transfer and power dissipation for the study of the production of pristinamycins by *Streptomyces pristinaespiralis* in shaking flasks

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ABSTRACT

Streptomyces pristinaespiralis is a filamentous bacterium used in the pharmaceutical industry for the production of pristinamycins. In previous works, it was shown that the occurrence of production and the antibiotics concentration could be related to gas–liquid transfer and power dissipation in shaking flasks. Nevertheless, in standard cultures, both mechanisms are coupled. It is then a difficult task to assign a precise physiological response to either oxygen transfer or power dissipation. The aim of the present study was to decouple the oxygen transfer coefficient (k_La) and the power dissipation per unit of volume (P/V) to study their respective impact on pristinamycin production. During cultures in flasks, the rotation diameter of the shaking table was changed to modify the k_La but not the power dissipation P/V. The influence of operating conditions with P/V ranging from 0.55 to 10.3 kW m^{-3} and k_La ranging from 30 to 490 h^{-1} have been determined on the microbial kinetics and also on the pellet diameters. The final biomass concentration and the release of antibiotics were then related to k_La, whereas the final pristinamycins concentrations, as well as the bacterial pellet diameter were mainly correlated to P/V independently of the k_La. The change in the pellet diameter could be the crucial parameter for the pristinamycins production as it might influence the nutriment transfer inside the pellets and the ratio of active cells in each pellet.

1. Introduction

Streptomyces pristinaespiralis, a filamentous Gram-positive bacterium, is industrially used for the production of pristinamycins [1,2] which depict a strong activity against methicillin-, penicillin-, and vancomycin-resistant bacteria, and a prolonged post-antibiotic effect [3,4]. Pristinamycins resistant pathogen strains are still rarely encountered [5,6]. Limitation of phosphate [7–9], nitrogen [10,11] and carbon [12,13] sources are well known as promoting antibiotics production. Beside these sources, as a result of its low solubility in culture media, oxygen is the most commonly limiting substrate in submerged culture. Moreover, oxygen uptake by the growing cells reduces the dissolved oxygen tension (DOT) in the medium, reaching thus, in some cases, critical values [14]. This can entail dramatic negative consequences on process performance [15–19]. One of the most common strategies to enhance oxygen mass transfer is the increase of the power dissipation per unit of volume (P/V), by an increase of the agitation rate. It is a key parameter in bioprocess engineering [20] as it influences heat and mass transfer, as well as mixing and circulation times [17,21,22]. Hence, it has been widely used as a bioreactor design and scale-up criterion [23]. However, not only gas–liquid but also liquid–biocatalyst mass transfers are also expected to be enhanced through increase of power dissipation. It is thus a hard task to study oxygen transfer effects on antibiotics production by only changing agitation levels. Moreover, increase of power dissipation also intensifies hydromechanical stress that could damage the cultured cells [16,24–29]. To better understand the respective influence of oxygenation and power dissipation on the antibiotic production by *Streptomyces* sp., it is thus important to consider these parameters independently. Studies dedicated to the decoupled effects of aeration and power dissipation on *Streptomyces* strains are very rare in literature. In bioreactor, using blended air/O_2 or N_2/O_2 streams at constant agitation rate, Yegneswaran et al. [19] have shown that an increase in DOT resulted in a higher concentration of cephamycin produced by *Streptomyces clavuligerus* independently of power dissipation.

Compared to other culture systems (mixed and sparged bioreactors), shaking flasks offer several advantages for the study of the

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influence of hydrodynamics on cell response such as single-phase and homogeneous hydrodynamics. Moreover, shaking flasks are appropriate in applications that require a high number of experiments, for example screening for efficient strains or optimization of media [30]. About 90% of all experiments are conducted in shaking flasks [31]. It is possible to measure online various parameters in shaking flasks like oxygen and carbon dioxide transfer rate [30,32], power consumption [33] and mixing time [34]. For these culture systems, oxygen is brought through the gas–liquid free surface, which entails a strong coupling of oxygen transfer with flask movement. However, Hansen et al. [35] recently showed that measurement of DOT in shaking flasks may be erroneous and that, under low filling volumes, optical spots may register oxygen concentrations in the gas phase of the head space of the flask or in the liquid film forming along the glass wall of the rotating flask. Thus, the preceding study justifies the use of OTR measurements as indicator of oxygen transfer capacities.

In previous studies [36,37], growth, induction of production as well as concentration of pristinamycins, power dissipation (P/V) and the gas–liquid mass transfer coefficient k_La have been correlated. But, as these last two parameters were coupled, it was difficult to precisely attribute the physiological responses to mixing or to oxygen transfer alone. As no study showing independent effect of P/V and oxygen transfer in shaking flasks on the antibiotic production by *Streptomyces* sp. can be found in literature, an original approach has been adopted to decouple these two phenomena. In the present study, the respective influences of P/V and k_La have been thus independently studied on biomass growth, diameter of pellets, consumption of substrates and occurrence as well as concentration of pristinamycins produced by *S. pristinaespiralis*.

2. Materials and methods

2.1. Microorganism and media

The strain used throughout this study was *S. pristinaespiralis* DSMZ 40338, a reference strain producing pristinamycins. The ICS complex medium was used for the germination of spores while the synthetic MPS2 medium was used for the culture of *S. pristinaespiralis*. Detailed about ICS and MPS2 composition can be found in Mehmood et al. [36].

2.2. Culture conditions

Precultures were inoculated with 1 mL of thawed spores calibrated at 3×10^7 Colony Forming Units mL^{-1} and poured into 250 mL unbaffled flasks containing 40 mL of ICS medium. The precultures were performed at 28 °C and 250 rpm on an orbital shaker with a 2.5 cm-shaking diameter. After 20 h, this preculture was used to inoculate MPS2 medium (5%, v/v) contained in 250, 500 or 1000 mL flasks. The dimensions of these flasks have been reported in Mehmood et al. [36]. The cultures were performed during 80 h, at 28 °C, either at 250 or 350 rpm and either on an orbital shaker with a 2.5 or 5 cm shaking diameter. Depending on the flask size, filling volumes varying between 10 and 200 mL were used. For offline analysis, several flasks with the same filling volume have been used so the flask used once for sampling was never put back on the shaking table.

2.3. Experimental measurements

2.3.1. Biomass

The dry biomass concentration was determined by absorbance at 660 nm according to the method described previously by Lubbe et al. [38] and by gravimetric method.

2.3.2. Rheology

The rheology of the culture broth was measured in a home-made rheo-reactor, consisting of a cylindrical vessel equipped with a double helical ribbon impeller installed in a controlled stress rheometer and according to the method described by Nzihou et al. [39].

2.3.3. Glutamate and arginine

Glutamate and arginine concentration were measured by HPLC (LC-20AD, Shimadzu France, Champs-sur-Marne, France) according to the method described previously [37].

2.3.4. Glucose

An automated chemistry analyzer (VITALAB Selectra 2, Merck, Darmstadt, Germany) was used to measure glucose concentrations by glucose oxidase assays.

2.3.5. Pristinamycins concentration

Pristinamycins were extracted from the culture medium and analyzed by HPLC (LC 10 AD-VP, Shimadzu, France) as previously described [2].

2.3.6. Pellet diameter

The diameter of microbial pellets was measured by laser diffraction technique (Mastersizer, Malvern, Worcestershire, UK). A helium–neon laser was used as radiation source and a series of detectors measured the light pattern produced. The lower and upper limit of obscuration was set at 10 and 30%, respectively. The optical properties of culture broth were assumed to be similar to those of water [40]. Diffraction data were collected by the detectors and post-treated by a dedicated software (Mastersizer, version 2.17).

2.3.7. Oxygen transfer rate (OTR)

The OTR to the culture was measured with RAMOS device using methods described by Anderlei and Büchs [30] and Anderlei et al. [32].

2.4. Hydrodynamics and mass transfer description

2.4.1. Measurement and calculation of power dissipation

Büchs et al. [33] have measured power dissipation in shaking flasks with various nominal volumes (from 100 to 2000 mL), filling volumes (from 4 to 20% of nominal volume), shaking frequencies (80–400 rpm) and at two shaking diameter of 2.5 and 5 cm. This study showed that P/V depended upon density and viscosity of culture medium, filling volumes, diameter of flasks, and agitation speed while at low liquid viscosity, the shaking diameter had no significant effect. The following relation has been proposed for the calculation of the power dissipation:

$$Ne' = 70Re^{-1} + 25Re^{-0.6} + 1.5Re^{-0.2} \quad (1)$$

where Ne' and Re are respectively the modified Newton and Reynolds numbers given by:

$$Ne' = \frac{P}{\rho N^3 d^4 V^{1/3}}, \quad Re = \frac{\rho N d^2}{\mu} \quad (2)$$

where P (W) is the power dissipation, ρ (kg m^{-3}) the liquid density, N (s^{-1}) the shaking frequency, d (m) the maximum flask diameter, V (m^3) the filling volume and μ (mPa s) the viscosity of culture medium.

Moreover, the power dissipation per unit of volume was experimentally measured for the set of experimental conditions given in Table 1 as proposed by Büchs et al. [33] and Büchs et al. [41]. Correlation (2) predictions were in agreement with the numerical simulations made by Mehmood et al. [36] and with experimental