Biochemical Engineering Journal 99 (2015) 167–176

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

Improvement of glucoamylase production using axial impellers with low power consumption and homogeneous mass transfer

Wenjun Tang [a], Ao Pan [a], Hongzhong Lu [a], Jianye Xia [a,**], Yingping Zhuang [a], Siliang Zhang [a], Ju Chu [a,*], Henk Noorman [b]

[a] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, PR China
[b] DSM Biotechnology Center, P.O. Box 1, 2600 MA Delft, The Netherlands

ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 22 March 2015
Accepted 30 March 2015
Available online 1 April 2015

Keywords:
Glucoamylase
Impeller type
Aspergillus niger
Morphology analysis
CFD

ABSTRACT

Significantly influenced by complex cell morphology, glucoamylase fermentation using *Aspergillus niger* is characterized by high apparent viscosity and shear-thinning rheology. In this study, the influence of liquid flow field patterns on morphology, broth rheology, mass transfer and glucoamylase production was investigated by applying two different configurations with radial (Ruston Turbine, RT) and axial (Wide-blade hydrofoil upward-pumping, WHu) flow impellers. It was found that empirical correlations for averaged quantities, such as the mass transfer coefficient and viscosity, cannot reasonably explain the observations. Therefore, numerical simulation was carried out to study the detailed characteristics of local field in lab-scale bioreactors. The results showed, under similar glucose and oxygen uptake rates, that the WHu configuration formed relatively homogeneous viscosity and mass transfer fields, while the RT configuration was accompanied with significant heterogeneities. Under these conditions, the fraction of active mycelia in pellets could be highly correlated with enzyme production, and a novel parameter (Active Part Percentage, APP) was defined to introduce the effects of flow field on pelletized morphology. The WHu impellers facilitated the formation of pellets and hairy structures, with a higher APP of the pellets. As a result, the culture with the axial flow impeller configuration exhibited a larger glucoamylase production rate (+25%) and product yield on sugar (+23%) and yield on energy (+60%) in comparison to the radial flow impeller. Computational fluid models were proposed to in-depth understand such results based on local mass transfer and viscosity values, since the average values are similar over the entire fermentation processes.

1. Introduction

Aspergillus niger, which has achieved GRAS status from FDA, is a valuable organism in the biotechnology industry because of its excellent ability in excreting large amount of organic acids and homologous as well as heterologous proteins [1].

During industrial fermentations with filamentous fungi like *A. niger*, monitoring and control of fungal morphology are difficult tasks. However, the cell morphology is a key performance factor, which to a great extent determines the broth rheological properties, mass transfer and mixing intensities. In a submerged cultivation process, fungi display either freely dispersed mycelia or dense pellets [2]. The fungal morphology with best productive performance varies from product to product and there is generally no unique preference which is the most suitable morphology [3–5]. For the production of glucoamylase by *A. niger*, the pellet is usually reported as being the desired morphology [6,7]. The morphology of *A. niger* is influenced by many factors including strain-specific properties, initial spore concentration, seed-cultivation, medium composition, dissolved oxygen concentration, pH, temperature, reactor scale, fermenter-type and power input [8–10]. Many strategies have been reported to control a proper fungal morphology, such as adding micro particles [2,11,12], adjusting broth osmolality [13,14] or changing the stirring speed or power input [15–17]. Changes in *A. niger* morphology associated with power input were studied in many cases [2,10,12,18,19], and revealed a delicate balance between generation and degeneration of hyphal structures.

For the fragmentation of pellets induced by agitation, three main mechanisms were summarized [20,21], i.e., interaction between

* Corresponding author at: State Key Laboratory of Bioreactor Engineering, East China University of Science & Technology, P.O. Box 329, 130 Meilong Road, Shanghai 200237, PR China. Fax: +86 21 64253702.
** Corresponding author.
E-mail addresses: jyxia@ecust.edu.cn (J. Xia), juchu@ecust.edu.cn (J. Chu).

http://dx.doi.org/10.1016/j.bej.2015.03.025
1369-703X/

Nomenclature

APP	Active part percentage (%)
C_D	Drag coefficient (−)
C_{pellet}	Pellet concentration (mL^{-1})
d_b	Diameter of air bubble (m)
D	Diameter of impeller (m)
D_L	Diffusion coefficient ($m^2\ s^{-1}$)
E	Elongation rate ($mm\ h^{-1}$)
E_O	Eötvös number (−)
EDCF	Energy dissipation/circulation function ($kW\ m^{-3}\ s^{-1}$)
$F_{D,lg}$	Drag force ($kg\ m^{-2}\ s^{-2}$)
F_{in}	Aeration rate ($mmol\ h^{-1}$)
g	Acceleration of gravity ($m\ s^{-2}$)
HGU	Hyphal growth unit (mm)
k	Geometrical factor of impeller (−)
k_L	Mass transfer coefficient ($m\ s^{-1}$)
K	Consistency index ($Pa\ s^n$)
M	Broth Weight (kg)
n	Flow index (−)
N	Agitation speed (s^{-1})
OUR	Oxygen uptake rate ($mmol\ kg^{-1}\ h^{-1}$)
OTR	Oxygen transfer rate ($mmol\ kg^{-1}\ h^{-1}$)
P	Power input (kW)
P_{OG}	Gassed power number of impeller (−)
PMV	Packed mycelium volume (−)
Q	Volumetric flow rate ($m^3\ s^{-1}$)
r_{core}	Core radius of a pellet (mm)
r_{crit}	Critical length of mass transfer in a pellet (mm)
r_{pellet}	Average radius of a pellet (mm)
R_e	Reynolds number (−)
$S_{project}$	Projected area of a pellet (mm^2)
t_c	Gassed circulation time (s)
u_i	Velocity of component i ($m\ s^{-1}$)
$v_{axial/radial}$	Axial/ radial component of liquid velocity ($m\ s^{-1}$)
v_b	Bubble slip velocity ($m\ s^{-1}$)
v_s	Superficial gas velocity ($m\ s^{-1}$)
V_L	Working volume (m^3)
$V_{pellet/inactive}$	Volume of a whole pellet/inactive part (mm^3)
W	Height of impeller (m)
$y_{i,in/out}$	The mole fraction of i in the inlet/outlet flow (−)

Greek letters

α_i	Volume fraction (−)
$\dot{\gamma}$	Average shear rate (s^{-1})
ϵ	Specific power input ($W\ kg^{-1}$)
λ_k	Kolmogorov length scale (m)
μ	Specific growth rate (h^{-1})
μ_{app}	Apparent viscosity (Pa s)
$\dot{\mu}_{app}$	Averaged apparent viscosity (Pa s)
ρ	Density of fluid ($kg\ m^{-3}$)
$\Delta\rho$	Density difference between liquid and gas ($kg\ m^{-3}$)
σ	Surface tension ($N\ m^{-1}$)

pellets and turbulent eddies, impact of impellers on pellets and collision among pellets. Since there is no big difference in density between microbial pellets and the surrounding fluid, the interaction between pellets and eddies is expected to be the most important mechanism of fragmentation [18]. As a consequence, knowledge of the flow characteristics in a stirred tank is crucial to design and operate a fungal fermentation process.

The characterization of pellet morphology has also been widely discussed in previous studies. A most simply way to judge the fungal pellet is to measure its diameter, projected area or concentration directly [8,22,23]. Other researchers used some calculated parameters like morphology number (MN) [24] or fractal dimensions [25] to give a more comprehensive evaluation on pellet morphology. Besides using optical microscopes, detailed structure information on a single pellet could also be obtained by other advanced techniques like focused beam reflectance measurement, laser diffraction, microelectrode and confocal laser scanning microscopy, etc. [26].

In this study, we have investigated a fungal fermentation process with two impeller configurations generating two distinct flow fields: one with traditional Rushton Turbine impellers, generating high shear rates and a non-uniform shear rate distribution, and the other with Wide-blade upward-pumping Hydrofoils, creating moderate shear rates and a more uniform distribution. The fungal pellet structure was further described via a newly-proposed morphological indicator, the active part percentage (APP), which quantifies the overall microbial activity of the pellets. The field information of the two impeller configurations were captured by the energy dissipation/circulation function (EDCF) [15,27] and the computational fluid dynamics (CFD) simulation [28]. This study showed that two distinct liquid flow patterns, with yet similar average flow characteristics, have a profound impact on enzyme productivity which is an important information for industrial scale-up.

2. Material and methods

2.1. Strain and spore preparation

A. niger CBS513.88 was initially cultured on a PDA (CM0139, Oxoid, UK) plate. A spore suspension was obtained by washing the plate cultures with a sterile aqueous solution of 0.01% Triton X-100 for further inoculation.

2.2. Medium and cultivation

The medium for the seed cultivation contained 20 g/L corn steep liquor and 22 g/L glucose·H_2O. The pH was adjusted to 6.5 using 3 mol/L NaOH prior to sterilization. A chemically defined medium was used for fed-batch fermentation. The composition contained 45.1 g/L glucose·H_2O, 10 g/L maltodextrin, 0.1 g/L $CaCl_2{\cdot}2H_2O$, 1.0 g/L $MgSO_4{\cdot}7H_2O$, 2 g/L critic acid, 3 g/L $(NH_4)_2SO_4$, 3 g/L KH_2PO_4, 1.5 g/L $NaH_2PO_4{\cdot}H_2O$, 0.04 g/L $MnSO_4{\cdot}H_2O$, 0.02 g/L $ZnCl_2$, 0.015 g/L $CuSO_4{\cdot}5H_2O$, 0.015 g/L $CoCl_2{\cdot}6H_2O$, 0.3 g/L $FeSO_4{\cdot}7H_2O$. The whole system was sterilized at 121 °C for 30 min. After that, the pH was adjusted and maintained at 4.5 using 12.5% (v/v) $NH_3{\cdot}H_2O$. The feed medium was exactly the same as the initial batch medium except for the concentration of glucose and maltodextrin was increased to 163.9 g/L and 50 g/L, respectively.

Seed cultivations were conducted in a 15 L stirred tank bioreactor with 8L working volume at 34 °C without pH control, inoculated with the spore suspension to a final concentration of about 10^6 spores/mL. After 24 h seed cultivation, 3 kg broth was transferred into a 50 L bioreactor with 30L working volume for further fed-batch. During the fermentation, air flow rate was kept at 1 vvm by a mass flow controller (Mass-Trak, Sierra, USA), glucose concentration was maintained at 5–10 g/L manually and pH is controlled at 4.5 using 12.5% (v/v) $NH_3{\cdot}H_2O$. The agitation speed was adjusted carefully to meet the requirement of oxygen demands in the batch phase and an oxygen-limitation strategy was applied afterwards [29].

Two different kinds of impeller configurations were applied in the 50 L bioreactor: a conventional set of 3 Rushton Turbines (RT) and a 3 Wide-blade upward-pumping Hydrofoil (WHu) impeller combination (for geometry information, see Fig. 1).