



Contents lists available at ScienceDirect

Bioresource Technology

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



Short Communication

Enhancing aspergiolide A production from a shear-sensitive and easy-foaming marine-derived filamentous fungus *Aspergillus glaucus* by oxygen carrier addition and impeller combination in a bioreactor

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ARTICLE INFO

Article history:

Received 19 July 2010

Received in revised form 9 October 2010

Accepted 11 October 2010

Available online 19 October 2010

Keywords:

Aspergillus glaucus

Aspergiolide A

Oxygen carrier

Shear-sensitive

ABSTRACT

Production enhancement of a novel antitumor compound aspergiolide A from shear-sensitive and easy-foaming marine-derived fungus *Aspergillus glaucus* HB1-19 in a 5-l stirred bioreactor was investigated. Two types of impellers, i.e., six-flat-blade disc turbine impeller (DT) and three-sector-blade pitched blade turbine impeller (PB) were used in this work. In cultures with fermentation medium, the combination of upper PB and lower DT led to the maximum dry biomass (13.8 g/l) and aspergiolide A production (19.3 mg/l). However, two PBs brought the highest aspergiolide A yield coefficient (1.9 mg/g dry biomass) despite it produced the lowest dry biomass (5.3 g/l). By contrast, two DTs and the upper DT and lower PB showed insignificant results. Feeding 0.35% (v/v) *n*-dodecane in cultures with upper PB and lower DT further improved aspergiolide A production by 31.0%, i.e., 25.3 mg/l, which is also 322% higher than that in the ordinary cultures with two DTs.

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1. Introduction

Aspergillus glaucus HB1-19 is a marine-derived filamentous fungus producing a novel antitumor compound aspergiolide A (Du et al., 2007). The compound has good bioactivity and the chemical and pharmacological study is in good progress. Accordingly, a large amount of aspergiolide A is needed currently.

Although great efforts were made to improve aspergiolide A production in shaking flask cultures (Cai et al., 2009; Sun et al., 2009, 2010; Tao et al., 2009), the shear-sensitive and easy-foaming characteristic of *A. glaucus* HB1-19 severely reduced the production in bioreactor cultures. It is generally agreed that agitation and oxygen provision are usually very important in filamentous fungi fermentation in stirred-tank bioreactors. For high viscous non-Newtonian broth caused by dispersed mycelia and clumps, a strong agitation is very necessary to improve mixing and oxygen transfer, whereas it could also initiate high shear stress which causes damage to microorganism and affects metabolite production (Elibol and Moreira, 2005; Jüsten et al., 1998).

This paper is to improve aspergiolide A production by introducing different impeller combinations and oxygen carrier in the bioreactor culture to reduce the shear stress and provide sufficient

oxygen. Moreover, as yet research about bioreactor culture optimization of marine microorganisms is deficient (Bhadury et al., 2006; Pomponi, 1999; Wagner-Döbler et al., 2002), this work also aimed to provide useful information for bioreactor cultures of marine filamentous fungi.

2. Methods

2.1. Fermentation system and culture conditions

Experiments were conducted in a 5-l stirred bioreactor (cylinder body of 153 mm inner diameter × 285 mm height and elliptic bottom of 153 mm inner diameter × 51 mm height) equipped with two layers of impellers (distance between impellers is 5 cm and lower impeller locates 5 cm above the vessel bottom). Two different types of impellers, i.e., six-flat-blade disc turbine impeller (DT, impeller diameter 65 mm, blade size 17 mm length × 13 mm height × width 1.5 mm) and three-sector-blade pitched blade turbine impeller (PB, impeller diameter 76 mm, blades angel 45° and width 1.5 mm, sector center angel 120°), and their combinations, i.e., two DTs, two PBs, upper DT and lower PB, upper PB and lower DT were applied in this work.

Fermentation medium is composed of 40 g soluble starch, 30 g maltose, 40 g sucrose, 2 g sodium glutamate, 1.07 g yeast extract paste, 1 g soybean powder, 2 g tryptone, 0.3 g MgSO₄·7H₂O and 0.5 g KH₂PO₄ per liter of artificial sea water. The artificial sea

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water, sporiparous medium and preculture medium were prepared as described before (Cai et al., 2009). Firstly, about 1×10^8 spores were inoculated into 100 ml preculture medium in a 500 ml Erlenmeyer flask and incubated at 28 °C and 180 rpm for 48 h, and then 420 ml precultures were transferred into 3 l fermentation medium and cultivated at 28 ± 0.2 °C. Antifoam was added with 0.02% (v/v) before inoculation and then replenished to 0.2% (v/v) during the culture process. For all batches, the air flow was 1.0 ± 0.1 vvm during the first 12 h, 1.7 ± 0.1 vvm during the next 108 h and then 0.25 ± 0.05 vvm until the end. The agitation speed was 300 rpm in the first 24 h and then maintained at 380 rpm during the following 96 h to enhance oxygen transfer. Afterwards, it was reduced to 300 rpm again and stayed until the end.

2.2. Analytical methods

The dissolved oxygen tension (DOT) was monitored by a polarographic probe (Oxyferm FDA, Hamilton, Switzerland) and the DOT of 100% was set as the constant DOT in the medium with aeration rate of 2 vvm and agitation speed of 600 rpm. Dry cell weight and aspergiolide A were analyzed as described before (Cai et al., 2009).

3. Results and discussion

3.1. Effects of different impeller combinations on growth, morphology and aspergiolide A production of *A. glaucus* HB1-19 in 5-l bioreactor

In the primary study of this work, the fungus produced a good quantity of aspergiolide A in shaking flask culture while very few in bioreactor culture. For filamentous fungi, shear stress caused by mechanical force usually destroyed their normal metabolisms. Therefore, glass beads were then used in the shaking flask cultures to primarily evaluate shear sensitivity of *A. glaucus* HB1-19. Our results showed that two glass beads (diameter 5 mm) added before inoculation decreased aspergiolide A production by 89.1% as compared to the control (72.3 ± 3.1 mg/l) and more beads caused even worse results, which indicated this fungus was very shear sensitive. Accordingly, different impellers combination were designed and applied in the bioreactor cultures (Fig. 1A–D). The maximum DCWs in the cultures using two DTs, two PBs, upper DT and lower PB, upper PB and lower DT were 8.0, 5.3, 8.0 and 13.8 g/l, and their highest aspergiolide A productions were 6.0, 9.9, 6.0 and 19.3 mg/l, respectively. Moreover, their highest yield coefficients of aspergiolide A production to cell growth ($Y_{P/X}$, aspergiolide A/DCW) were 0.8, 1.9, 0.8 and 1.4 mg/g DCW, respectively. Overall, two PBs could

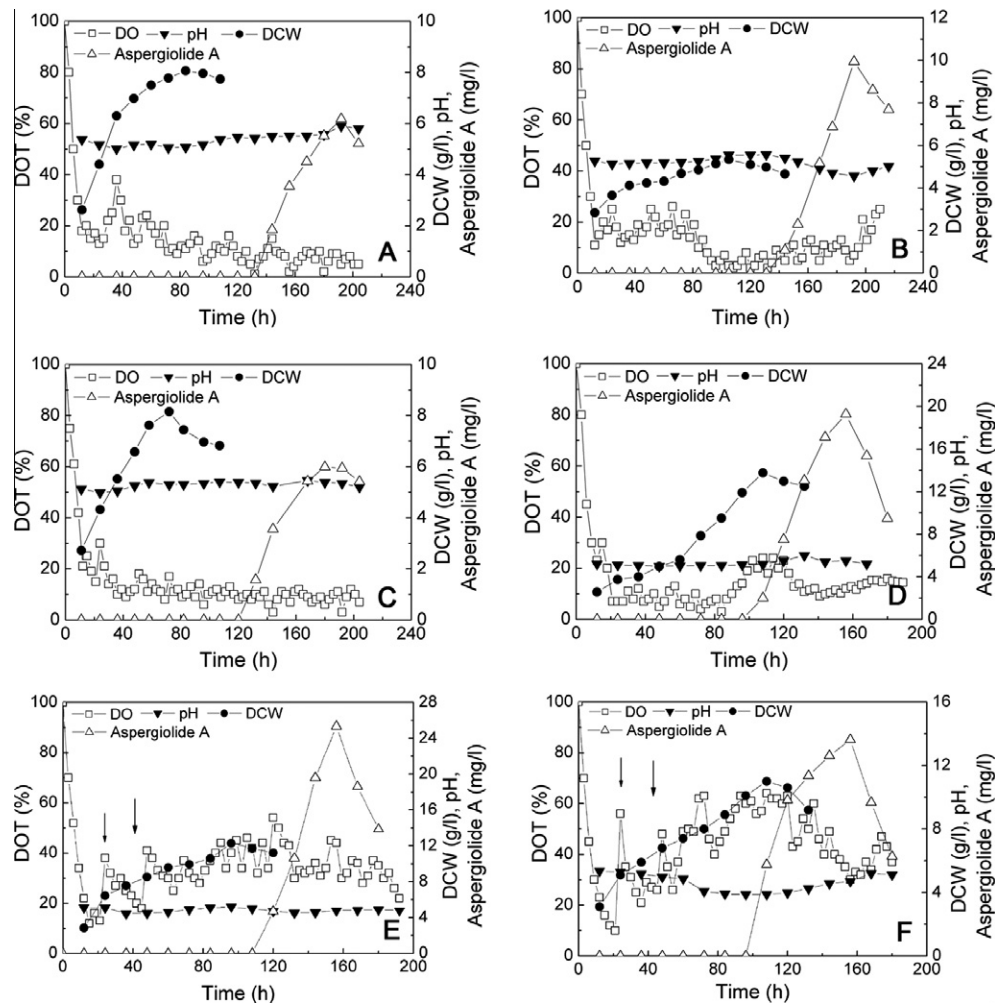


Fig. 1. Time courses of the 5-l stirred-tank bioreactor cultures of *A. glaucus* HB1-19 with different impeller combinations. (A) two DTs; (B) two PBs; (C) upper DT and lower PB; (D) upper PB and lower DT; (E) upper PB and lower DT with 0.35% (v/v) *n*-dodecane addition; (F) upper PB and lower DT with 0.58% (v/v) *n*-dodecane addition. The arrows in E and F indicated two equal feedings of *n*-dodecane. DT represents six-flat-blade disc turbine impeller and PB represents three-sector-blade pitched blade turbine impeller.

improve aspergiolide A productivity despite it was unfavorable for cell growth but upper PB and lower DT improved both. Contrarily, two DTs and upper DT and lower PB were unfavorable to either of them. As we know, the disk turbine impeller generates radial flow, strong gas dispersion and great shear stress, whereas the pitched blade impeller produces axial flow, small shear force and good micromixing (Kumaresan and Joshi, 2006; Papagianni, 2004). Therefore, a lower DT was more beneficial for bubble broken up with air admission from the bottom sparger. Then with strong axial flow by upper PB, it would provide better living conditions of abundant gas distribution, uniform macromixing and low shear force for *A. glaucus* HB 1-19 in the culture.

The fungal morphologies in various cultures greatly differed from each other. Freely dispersed clumps and highly branched hyphae always came with two DTs, whereas the two PBs promoted formation of clumps with very slender hypha. Mycelia from cultures under other two conditions were also different and more related to the lower impeller. All the results indicated that large clumps consisted of relatively long mycelia were better for cell growth and aspergiolide A production.

3.2. Effects of *n*-dodecane on growth, morphology and aspergiolide A production of *A. glaucus* HB1-19 in 5-l bioreactor with upper PB and lower DT impellers

Dissolved oxygen tension (DOT) is also an important limit factor in filamentous fungi cultures and it evidently affected aspergiolide A production in shaking flask cultures of *A. glaucus* HB 1-19 (data not shown). However, DOT could not be enhanced by increasing the agitation speed because the fungus is shear-sensitive, or enlarging the air flow because of its easy-foaming character. *n*-Dodecane, a non-aqueous oxygen carrier, was proven to highly promote oxygen transfer rate. It was reported that addition of 5% (v/v) *n*-dodecane could stimulate mycelial growth of *Aspergillus niger*, enhance citric acid accumulation but have no adverse effects on the cells (Wang, 2000). Here, on the basis of the above *A. glaucus* HB 1-19 bioreactor cultures using upper PB and lower DT, *n*-dodecane was then applied to further enhance aspergiolide A production.

The DOT rose up sharply after *n*-dodecane addition (0.35%, v/v) and stayed at higher levels than the control (Fig. 1E vs. Fig. 1D). The highest aspergiolide A production was 25.3 mg/l, which was 31.0% higher than the control. However, the maximum DCW decreased 12.1% as compared to the control of 13.8 g/l. For *n*-dodecane addition of 0.58% (v/v), the DOT was much higher than both the control and the 0.35% (v/v) *n*-dodecane addition culture. The maximum DCW and aspergiolide A production were 20.0% and 29.6% lower than the control, respectively (Fig. 1F vs. Fig. 1D). For morphology, it tended to be pellets with DOT increase and higher DOT promoted the pellets more compacted. Overall, aspergiolide A production favored a relatively high DOT but was inhibited by excess oxygen provision.

Although different impellers and oxygen carriers have been investigated in microorganism cultures, few cases involved their combined use. In this work, we combined them together and diminished the harm of shear-sensitive and easy-foaming problems in *A. glaucus* HB1-19 bioreactor culture successfully. As oxygen carriers are usually stable, they are working during the whole culture process and their combined use with agitation

optimization will be very useful in either lab-scale or large-scale fermentation of shear-sensitive and easy-foaming microorganisms. Moreover, this work might also provide useful information for the ever increasing marine fungi cultures.

4. Conclusions

In 5-l bioreactor, the impeller combination of upper PB and lower DT was proven to be the optimal impeller combination for both mycelia growth and aspergiolide A production despite the productivity was lower than two PBs. Feeding 0.35% (v/v) *n*-dodecane further enhanced aspergiolide A production by 31% but decreased mycelia growth by 12.1%. By employing impeller combinations and oxygen carrier additions, aspergiolide A production was increased by 322% as compared to the common cultures with two DTs. This work offer enlightenment and reference certainly to shear-sensitive and easy-foaming bioreactor fermentation and marine filamentous fungi culture.

Acknowledgements

This work was supported by the National High Technology Research and Development Program of China (2010AA09Z429), Shanghai Rising-Star Program (09QA1401600) and Shanghai Leading Academic Discipline Project (B505). We thank Ocean University of China for supply of the strain and the aspergiolide A standard.

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