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Full Length Research Paper

Cell number as an important variable in optimising inoculum age and size in yeast cultivation

Zhenqiang Xia and Shengjun Wu*

School of Marine Science and Technology, Huaihai Institute of Technology, 59 Cangwu Road, Lianyungang, 222005, China.

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The growth lag phase during microbial fermentation reduced productivity and equipment efficiency. Inoculum age and size were the two important variables considered in fermentation optimisation. Previous studies investigated these two variables separately, in contrast to the orthogonal or use of response surface modelling method by recent papers. In nearly all cases, the inoculum from the late exponential or early stationary phase was selected as optimal. In the current study, the authors optimised inoculum conditions using a strategy that combined inoculum age and size as inoculum cell number to shorten the lag phase in yeast cultivation. Inoculum from the middle exponential phase (7th h) exhibited priority in activity and adaptability. This condition was confirmed by inoculating the resuspended cell sediments into fresh potato dextrose medium. The results indicate that biomass (cell number for single cell microbes and wet/dry cell weight for filamentous strains), rather than inoculum age and size, should be considered during inoculum condition optimisation.

Key words: Fermentation, optimisation, cell number, exponential phase, inoculum age, inoculum size.

INTRODUCTION

Inoculation of cells into a complete fermentable growth medium elicits a delay or pause of growth known as the growth lag phase (Ma et al., 1997). Many studies have attempted to shorten the lag phase and promote productivity. Several studies have focused on the addition of certain amino acids (Machlis, 1957) or the removal of inhibitory metabolite by electrodialysis (Yamamoto et al., 1993). However, majority of these studies were performed by comparing different aged inocula to obtain the inoculum with the shortest lag phase during the fermentation stage. In previous papers, inoculum age and size were investigated separately, on the enhancement of milbemycin yield (Warr et al, 1996) and on lactic acid production (Martinkova et al, 1991). Some researchers infer that inoculum age and size are twin parameters, that is, they are interrelated and should be optimised together to deter-mine the optimum period and biomass density for inoculation. Sen and Swaminathan (2004) used computer-aided response surface modelling, optimisation and analysis of the age and size of the two-stage inocula in a batch reactor to improve surfactin production. Dasu and Panda (2000) used the central composite design to determine the optimal levels of inoculum age, size and slant age. Fermentation time of of *Penicillium griseofulvum* MTCC 1898 and MTCC 2004 decreased by 4 and 2 days, respectively. In nearly all cases, the inoculum from the late exponential or early stationary phase was selected as optimal. However, cell concentration varies sharply with inoculum age, and the priority in the inocula with larger cell numbers does not mean a priority in cell activity and adaptability. In the current study, a strategy that combines inoculum age and size as the inoculum cell number is used in optimising inoculum conditions to shorten the lag phase in yeast cultivation. The result is quite different from that in con-ventional concepts.

*Corresponding author. E-mail: wushengjun008@sina.com. Tel: +86 518 85865427. Fax: +86 518 85865428.

MATERIALS AND METHODS

Saccharomyces cerevisiae isolated from active dry yeast (Angel Yeast Co., Ltd. 168 Chengdong Avenue, Yichang, Hubei, P. R. China. 443003) was used throughout the study. Stock cultures were maintained on potato dextrose agar (PDA) at 4°C and subcultured every month.

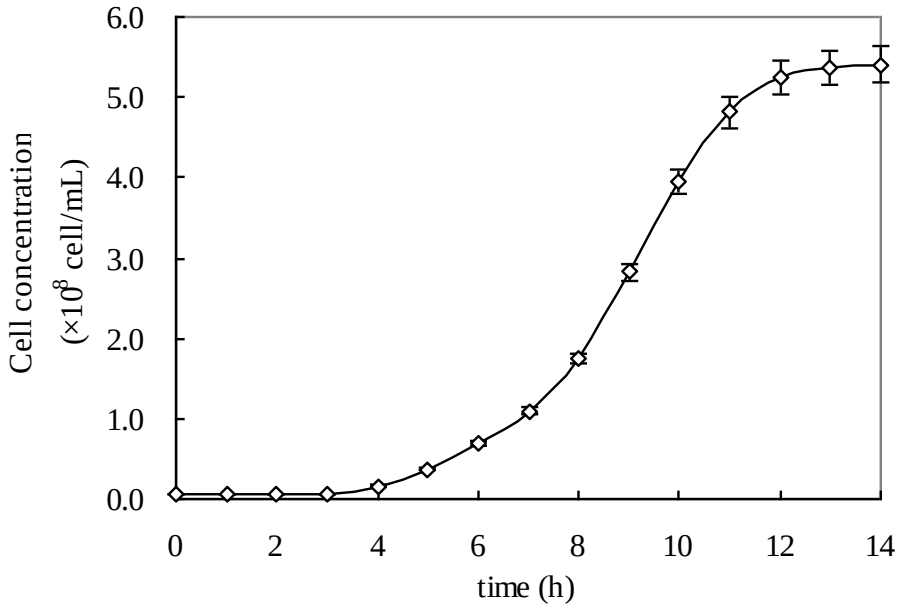

Figure 1. Time course of *S. cerevisiae* cell growth. Data are shown as mean ± SD (n = 6).

Preparation of inoculum

Sterile PDA medium (100 ml) in a 500 ml Erlenmeyer flask was inoculated with two loops of cells from an overnight agar slant and subsequently incubated at 28°C with shaking at 180 rev/min. After 7 to 11 h incubation, the culture was inoculated in a fresh medium for fermentation.

Cultivation

Sterile PDA medium (100 ml) in 500 ml Erlenmeyer flask was inoculated with liquid inoculum or cell suspension and subsequently incubated at 28°C with shaking at 180 rev/min.

Inoculum cell number optimisation in yeast cultivation

Yeast cell concentrations of the culture incubated for 7, 9 and 11 h were determined. Different inoculum sizes corresponding to each inoculum age was inoculated unto fresh medium to keep the inoculum cell numbers identical. Cell growth was monitored at 9 h.

Comparison of yeast cell adaptability

Broths (100 ml) incubated for 7 and 11 h were centrifuged at 3500 ×*g* for 10 min to obtain the pure cell sediment and cell-free supernate, respectively. The sedimentary cells were resuspended in 10 ml sterile saline water. After determining the cell concentration, the suspensions were inoculated in 100 ml PDA medium in 500 ml shake flasks at a concentration of 1×10^7 cell/ml. Then, the suspensions were incubated at 28°C with shaking at 180 rev/min to investigate the growth rate.

Investigation of supernate

The broth (100 ml) incubated for 7 h was centrifuged at 3500 ×*g* for 10 min. The sedimentary cells were resuspended in 10 ml sterile saline water. After determining the cell concentration, the suspension was inoculated again in 100 ml supernate and fresh PDA medium in 500 ml shake flasks at a concentration of 1×10^7 cell/ml each. Then, the mixture was incubated at 28°C with shaking at 180 rev/min to investigate the growth rate.

Analytic methods

Yeast cell number in the inoculum or broth was determined hourly by a haemocytometer. After inoculation, the cells were considered to be entering the growth exponential phase if the cell number continuously increased by more than 40% within 1 h.

RESULTS AND DISCUSSION

Yeast cell growth curve

The growth curve of *S. cerevisiae* in a 500 ml shake flask containing 100 ml PDA medium was observed (Figure 1). With inoculum from slant agar, the cells entered the exponential phase at 4 h and the stationary phase at 12 h.

Inoculum age and size optimisation using cell number

Three levels of inoculum cell numbers were investigated (Table 1). The inoculum obtained from the 11th h grew fastest when the inoculum sizes were identical. By contrast, inoculum obtained from the 7th h grew faster than that from the 11th h when the cell numbers were identical. As a result, inoculum from the 7th h with an

Table 1. Effects of inoculum cell number as a combination of inoculum age and size on cell growth[a].

Inoculum age (h)	Cell concertration (×10^8 cell/ml)	Inoculum volume (ml)	Inuculum cell number (×10^8 cell)	Cell concentration at 9 h in fermentation broth (×10^8 cell/ml)
7.00	1.095±0.042	4.5	4.928±0.091	1.824±0.028
		9.0	9.855±0.332	3.900±0.065
		13.5	14.783±0.405	4.942±0.107
9.00	2.820±0.069	1.8	5.076±0.158	1.573±0.037
		3.5	9.870±0.211	3.355±0.089
		5.0	14.100±0.375	4.231±0.083
11.00	4.817±0.085	1.0	4.817±0.072	1.369±0.042
		2.0	9.634±0.197	2.550±0.074
		3.0	14.451±0.377	3.589±0.126

[a] Mean ± S.D. from three independent experiments.

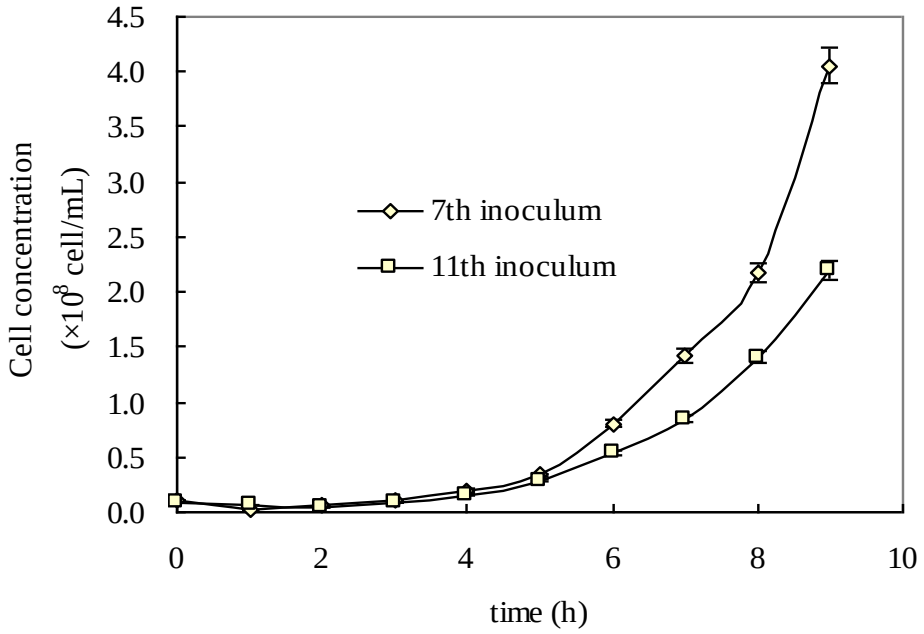

Figure 2. Time courses of cell concentration in fermentation broth inoculated with resuspended cells from 7th and 11th inoculum. Data are shown as mean ± SD (n = 6).

inoculum size of 13.5 ml grew fastest. However, a high inoculating rate (13.5%) was needed.

Cell adaptability investigation

The resuspended yeast cells from the 7th h broth grew faster than those from the 11th h broth (Figure 2). This phenomenon indicates that yeast cells from the middle exponential phase are more active and adaptative to fresh medium than those from the late exponential phase. However, the latter had a higher cell concentration in the broth.

Comparison of fresh medium with supernate from the 7th h broth

A lag phase lasting for approximately 1 h still existed even after inoculation in supernate, although the supernate was infact a familiar environment to the sedimentary cells (Figure 3). However, unlike the evident decrease in cell number in the fresh medium, the cell number in the supernate remained unchanged during the lag phase. Cells grew faster in the supernate than in the fresh medium, but the growth rate decreased after 6 h. This condition may have been caused by the insufficiency of nutrients as a result of the consumption during inoculum cultivation.

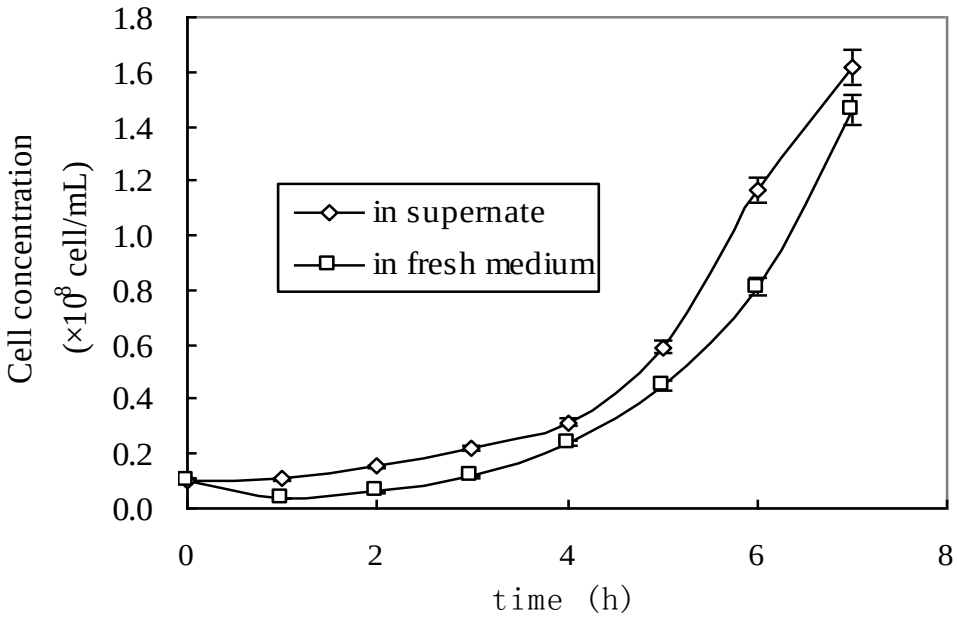

Figure 3. Time courses of cell concentration in fresh medium and supernate with identical inocula. Data are shown as mean ± SD (n = 6).

This phenomenon indicates that although some metabolites secreted into the inoculum by yeast cells are in favour of shortening the lag phase, certain nutrients should be added if the inoculum size is quite large.

Conclusions

In the current study, the cell number was used as a combined and pioneer variable in optimising inoculum age and size in yeast cultivation. Yeast cells from the mid-exponential phase (7^{th} h) and an inoculum size of 13.5% adapted faster to a fresh medium than older cells (9^{th} and 11^{th} h), and resulted in the shortest lag phase. These results are consistent with the study of Ginovart et al., (2011) in which the shortest lag phase and the time until the first division are reportedly obtained with the largest inocula and the youngest inoculated parent cells. Certain metabolites in the supernate were found to be in favour of the adaption of the cell to its environment.

Whether this inoculum-optimising strategy is suitable for fermentation by filamentous microbes, such as actinomycetes and fungi (in which the wet/dry cell weight, instead of cell number), is a significant topic that should be investigated. The effect of the proposed optimising strategy on the yield of primary and secondary metabolites must be explored further.

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