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THE INTER-RELATIONSHIP BETWEEN INOCULUM CONCENTRATION, MORPHOLOGY, RHEOLOGY AND ERYTHROMYCIN PRODUCTIVITY IN SUBMERGED CULTIVATION OF *Saccharopolyspora erythraea*

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Abstract - Submerged cultivation of *Saccharopolyspora erythraea*, at different initial spore concentrations, was carried out to study the inter-relationship between inoculum concentration, morphology, rheology and erythromycin production. Pellet morphology was dominant in runs at 10^3 and 10^4 spore/ml initial spore concentrations, whereas there was a significant presence of clump morphology in runs at initial spore concentrations of 10^5-10^7 spore/ml. The *S. erythraea* cultivation broths exhibited Newtonian rheology in runs at initial spore concentrations of 10^3 and 10^4 spore/ml, whereas at higher initial spore concentrations the rheological data could be fitted with the power law model. Runs in which clump morphology was predominant resulted in the highest erythromycin productivities. The findings of the present work suggest that the predominance of clump morphology, smaller sized clumps and, in the case of non-Newtonian *S. erythraea* cultivation broths, a decrease in viscosity enhance erythromycin production.
Keywords: Erythromycin; *Saccharopolyspora erythraea*; Morphology; Inoculum spore concentration.

INTRODUCTION

The information available about the morphology of actinomycetes grown in submerged culture is restricted, even though this group of microorganisms is used to produce many of the antibiotics in current use (Whitaker, 1992). The morphology of these filamentous microorganisms is an important factor when they are employed in the industrial production of secondary metabolites, such as antibiotics. Control of mycelial morphology is often regarded as a prerequisite to ensure increased productivities in industrial applications (Papagianni and Mattey, 2006). For example, a mycelial form of *Streptomyces griseus* is required for streptomycin production, but pelleted and fragmented forms are undesirable (Schatz and Waksman, 1945). On the other hand, pellets of *S. nigrificans* have been shown to produce

*To whom correspondence should be addressed more glucose isomerase than the mycelial form (Whitaker, 1992).

One of the factors that has been reported to affect the morphology of filamentous microorganisms is the inoculum size (Whitaker, 1992; (Glazebrook and Vining, 1992; Lawton *et al*., 1989; Smith and Calam, 1980; Tucker and Thomas, 1992; Tucker and Thomas, 1994). An inoculum with high concentration of viable spores tends to produce a disperse form of growth, whereas the use of an inoculum with low spore concentration normally results in pellet formation (Whitaker, 1992). The inoculum spore concentration, in which the transition from predominantly pellet to a predominantly clump or dispersed mycelial form occurs, depends on the strain and can be affected by nutrient concentrations and other fermentation conditions (Braun and Vecht-Lifshitz, 1991).

The effect of spore concentration on the morphology of filamentous fungi has been extensively investigated (Smith and Calam, 1980; Tucker and Thomas, 1992; Du *et al*., 2003), whereas there is less information for actinomycetes. For these types of bacteria the inoculum size has been reported to affect the degree of fragmentation of hyphae (Tresner *et al*., 1967), the mycelial aggregate size (Glazebrook and Vining, 1992) and the average pellet size (Vecht-Lifshitz *et al*., 1990), although in some cases the morphology has been reported not to be strongly affected by the inoculum size (Glazebrook and Vining, 1992).

Saccharopolyspora erythraea is an industrially important actinomycete used for the production of erythromycin. The effect of various parameters such as shear, biomass concentration and medium components (Bushell *et al*., 1997a; Hamedi *et al*., 2004; Heydarian *et al*., 1999; Rostamza *et al*., 2008; Sarra *et al*., 1996) on the morphology of this actinomycete has been previously reported. To the knowledge of the authors, there is no report in the literature about the effect of inoculum size on the morphology, rheology and erythromycin production by this microorganism; furthermore, the relative erythromycin productivities of pellet and clump forms of this actinomycete have not been considered in any previous study.

In this study, the effect of the morphology of a *S. erythraea* culture on the production of erythromycin in shake flask cultivation was investigated. A change in morphology was achieved by varying the initial spore concentration in the range of 10^3-10^7 spore/mL. Morphological and rheological parameters were characterized in order to gain a better insight into the inter-relationship between *S. erythraea* morphology, rheology of the cultivation broth and erythromycin productivity in shake flask cultivation.

MATERIALS AND METHODS

Microorganism and Inoculation

The culture used throughout this study was a mutant strain of *Saccharopolyspora erythraea* (NUR001) provided by the Shafa-e-Sari Pharmaceutical Co., Tehran, Iran (Hamedi *et al*, 2002). The strain was stored as a spore suspension at -20°C. Spore suspensions were prepared by initially incubating agar slants of *S. erythraea* at 30°C for 10-14 day. The surface of the slants were then scoured with a solution containing 20% (v/v) glycerol and 0.1% (v/v) Tween 80 and the resulting spore suspension was subsequently passed through sterilized cotton. The harvested spores were pooled and stored at -20°C. The concentration of spores in the suspension was determined by the spread plate method. This was done by plating the spore suspension for 48 hours at 30°C in nutrient agar medium and determining the spore concentration using the plate count method.

500 mL Erlenmeyer flasks containing 200 mL of fermentation medium were inoculated with appropriate volumes of spore suspensions to obtain initial spore concentrations in the range of 10^3-10^7 spore/mL and incubated at 30°C with shaking at 200 rpm. All experiments were performed in duplicate.

Growth Conditions

The stock culture was maintained on agar slants containing the following nutrient (in g/L): 10 corn steep liquor, 10 starch, 2.5 $CaCO_3$, 3 $(NH_3)_2SO_4$, 3 NaCl, 20 agar and 2 mL microelement solution. Microelement solution had the following composition (in g/L): 100 $MgSO_4 \cdot 7H_2O$, 2 $FeSO_4 \cdot 7H_2O$, 2 $ZnSO_4 \cdot 7H_2O$, 0.4 $CuSO_4 \cdot 5H2O$, 0.1 $CoCl_2 \cdot 6H_2O$, and 0.1 % (v/v) HCl. The fermentation medium composition was as follows (in g/L): 30 glucose, 6 yeast extract, 4 bacteriological peptone, 2 glycine, 0.5 $MgSO_4 \cdot H_2O$ and 0.68 KH_2PO_4 (Heydarian *et al*., 1999). The initial medium pH was adjusted to $pH=7\pm0.1$. Glucose and KH_2PO_4 were separately sterilized and aseptically added to the sterile medium. Corn steep liquor and starch were obtained from Shafa-e-Sari Pharmaceutical Co.

Morphological Measurements

A semi-automatic image analysis method was used to characterize the morphology of *S. erythraea* in samples taken from the shake flasks at the same time each day. In this method, a Bel optical microscope (model BIO2-Video, Bel Photonics, Monza (Milan), Italy) with video output connected to a computer monitor was used to take video clips from slides prepared from appropriately diluted and stained samples. The clips were then processed to obtain between 100-300 picture frames for each slide. The number and dimensions of pellets, clumps and free mycelia were determined manually. The morphological parameters measured in this study for these three growth forms are described in Table 1 (Heydarian *et al*., 1999; Rodríguez Porcel *et al*., 2005). For each parameter, the values reported are the mean of between 100-300 measurements.

Biomass Determination

Microbial dry cell weights (DCW) were determined by filtering 5 mL samples through predried and weighted membrane filters (Millipore, 0.45 μm). The filter was then rinsed with distilled water (2×5 mL) prior to drying in an oven at 100°C for 24 h. The results presented are the mean of DCW obtained in two separate cultivations.

Erythromycin Measurements

The total erythromycin concentration in the samples taken from shake flasks was measured as follows (Hamedi *et al*., 2002): After centrifugation, the cell-free broth was diluted with 0.2 M carbonate/bicarbonate buffer, pH 9.6, and then extracted with an equal volume of chloroform. Extracted erythromycin was mixed with Bromophenol Blue reagent. The absorbance of the separated organic phase was measured at 415 nm. The results presented are the mean of total erythromycin concentrations obtained in two separate cultivations.

Rheological Measurements

The rheological properties of *S. erythraea* fermentation broths were characterized using a Haake rheometer (Model CV100) employing a concentric cylinder sensor system (type ZB15). The measurements were carried out over a shear rate range of 0.3–300 s^{-1}, at room temperature.

RESULTS AND DISCUSSION

Figure 1 shows the morphological population balances of *S. erythraea* in shake flask cultures for various initial spore concentrations (10^3-10^7 spore/mL) as a function of cultivation time. The results showed the existence of three morphological growth forms of *S. erythraea* (NUR001) at different cultivation times and for various initial spore concentrations, namely pellet, clump and free dispersed mycelium. These different growth forms are illustrated in Figure 2. However, the proportion of each growth form in the culture media was found to depend on both the time of cultivation and the initial spore concentrations. The results show that, with an increase in initial spore concentration, the proportion of *S. erythraea* cells exhibiting clump morphology increases; pellet morphology is dominant at initial spore concentrations of 10^3 and 10^4 spore/mL (Figures 1a and 1b), whereas at 10^6 and 10^7 spore/mL clumps are the predominant morphology (Figures 1d and 1e). The results at 10^6 spore/mL initial spore concentration are in line with a previous report by Heydarian *et al*. (1997) for another strain of *S. erythraea*. An increase in the proportion of clump/dispersed growth morphology with an increase in initial spore concentration has also been reported for other filamentous actinomycetes (Whitaker, 1992; El-Enshasy *et al*., 2000).

Table 1: The morphological parameters of *S. erythraea* measured by image analysis

Morphology	Morphological parameters	Description
Pellet	Pellet core diameter	Equivalent diameter of the measured core area (central compact core region)
Pellet	Mean hairy length of pellet	The width of the hairy zone (peripheral filamentous or hairy region)
Mycelial clump	Mean major axis	Maximum mycelia diameter

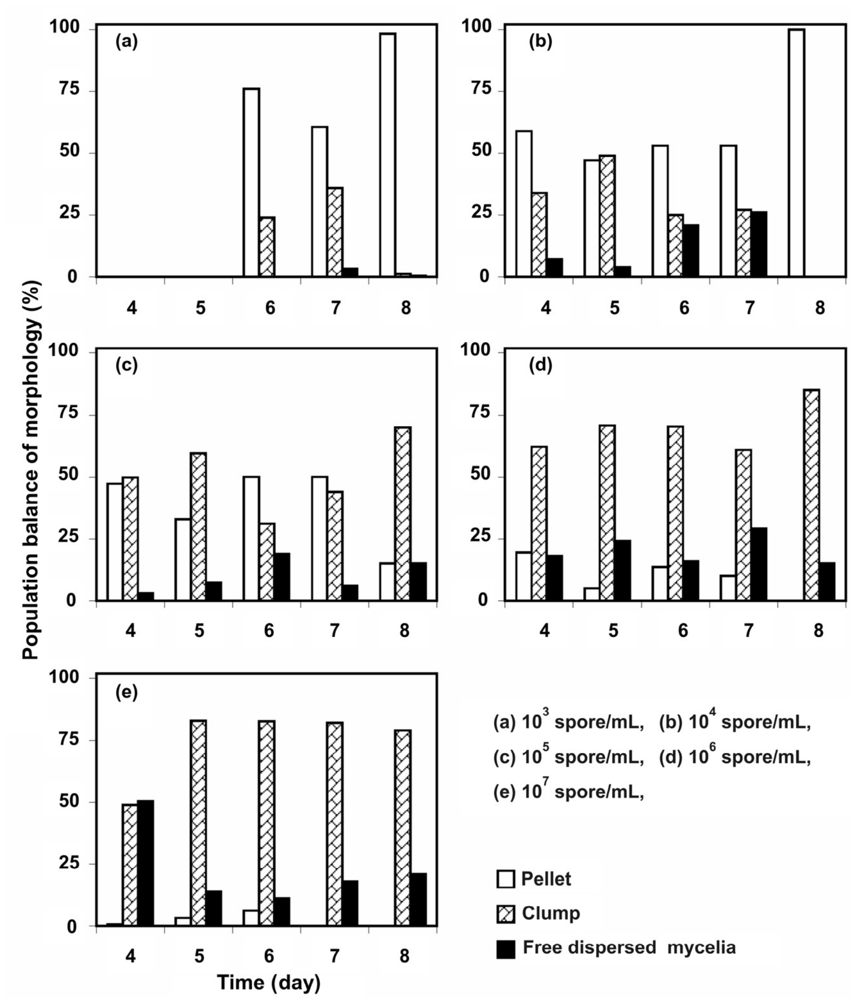


Figure 1: The effect of the initial spore concentration on the proportion of morphological growth forms in cultures of *S. erythraea* (NUR001) at different cultivation times

(a) **(b)** **(c)**

Figure 2: Examples of typical *S. erythraea* (NUR001) morphology: (a) free dispersed mycelium; (b) clump; (c) pellet

The rheological analysis indicated that, in the runs at 10^3 and 10^4 spore/mL initial spore concentration - in which pellet was the dominant morphology the cultivation broth exhibited Newtonian characteristics (Figure 3a). With the increase in the concentration of clumps/dispersed mycelia with further increases in initial spore concentration, the cultivation broth became non-Newtonian and the rheological data could be fitted to the power law model ($\tau = K\gamma^n$) with $R^2>0.999$ (results not presented). In the case of runs at an initial spore concentration of 10^5 spore/mL, the value of n was near 1(results not presented). For runs with initial spore concentrations of 10^6 and 10^7 spore/mL (Figure 3b), the consistency index (K) increased, whereas the flow behavior (n) decreased with the increase in the proportion of clump/dispersed morphology, indicating that *S. erythraea* cultivation broth becomes more non-Newtonian under these conditions. Also, as the cultivation proceeded, K decreased and n increased, indicating that the cultivation broth becomes less non-Newtonian with time.

The morphological data presented in Figure 4 shows that the mean major axis of the clumps decreased from day 4-5 onwards and was lower for runs at 10^6 spore/ml initial spore concentration compared to the runs at 10^5 and 10^7 spore/ml. Also, the change in the rheological parameters can be roughly correlated with the trend of change in mean major axis of the clump. For example, in the runs at 10^6 spore/ml initial spore concentration there is a sharp change in K and n at day 6, with the values remaining fairly constant thereafter; this corresponds to a sharp drop in mean major axis of the clump at the same day. On the other hand, for runs at 10^7 spore/ml initial spore concentration, K and n show a gradual change with time during cultivation (Figure 3b) that correlates with a gradual drop in the mean major axis of the clumps (Figure 4). The trend of change in dimensions of the clumps is also illustrated pictorially in Figure 5 for runs at 10^7 spore/mL initial spore concentration.

The morphological data for cultivations, in which either pellet was the dominant morphological form or was present in a significant amount, is presented in Figure 6. The results suggest an increase in pellet size with cultivation time and a subsequent decrease as a result of pellet fragmentation when the cultivation is allowed to proceed long enough (Figure 6a). The maximum pellet size attained during *S. erythraea* cultivation was the highest for runs at 10^3 spore/mL initial spore concentration; the maximum pellet sizes achieved at the two higher initial spore concentration were fairly similar to each other. A decrease in pellet size with an increase in inoculum level has been previously reported for a *Streptomyces* sp. for initial spore concentrations in the range 10^2-10^6 spore/mL (Dobson *et al*., 2008). For cultures of *Streptomyces griseus*, Kim and Hancock (2000) reported a similar trend for inoculum concentration in the range 1.5×10^4 to 1.5×10^6 spore/mL.

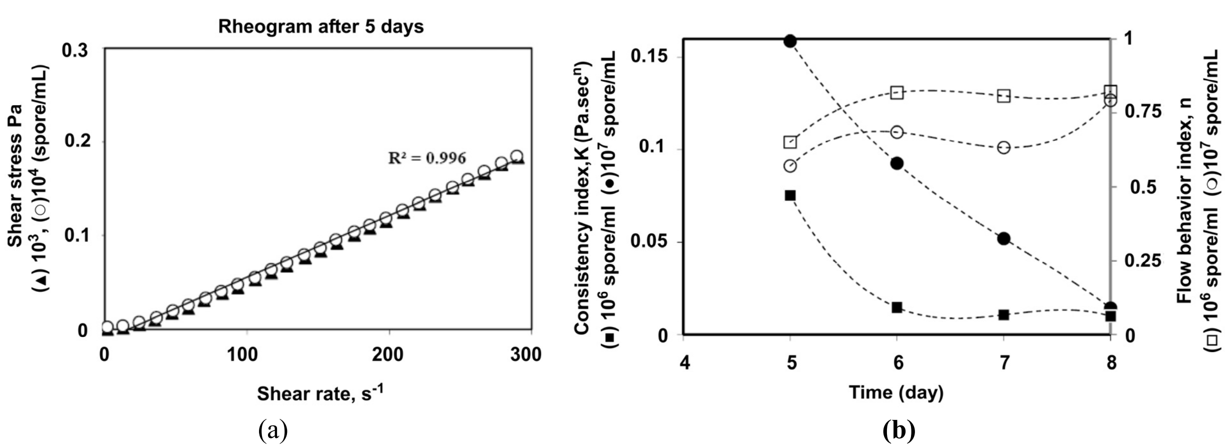


Figure 3: (a) Newtonian model curve fitting for rheological data of *S. erythraea* fermentation (Initial spore concentration = 10^3 and 10^4 spore/mL), (b) Profiles of consistency index (K) and flow behavior index (n) with cultivation time of *S. erythraea* fermentation (Initial spore concentration = 10^6 and 10^7 spore/mL)

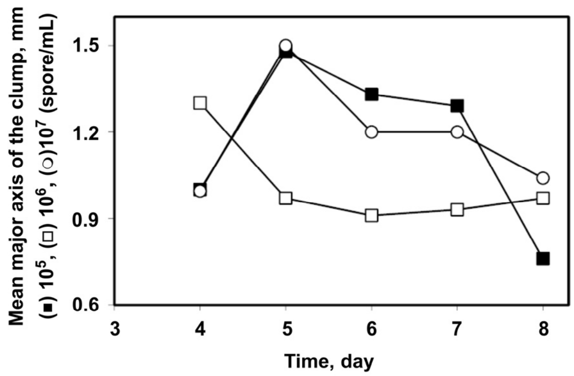


Figure 4: Variation in the mean major axis of the clump as a function of cultivation time for initial spore concentrations of 10^5, 10^6 and 10^7 spore/mL

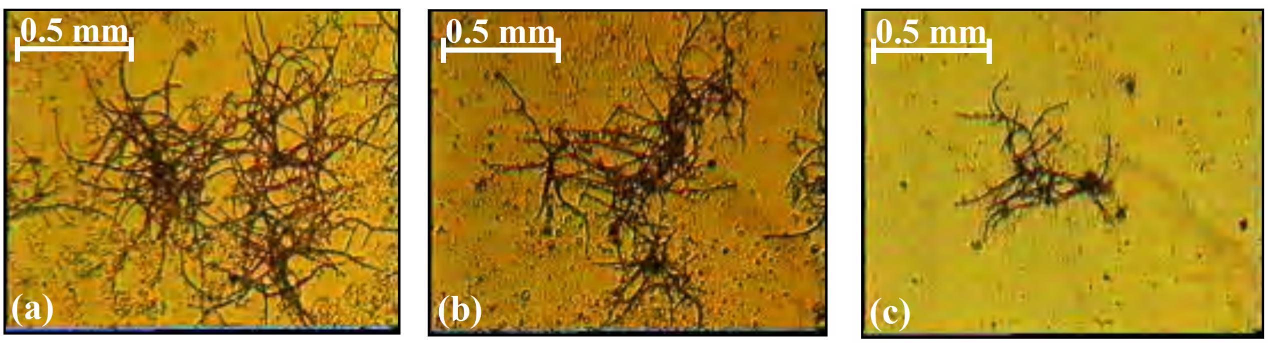
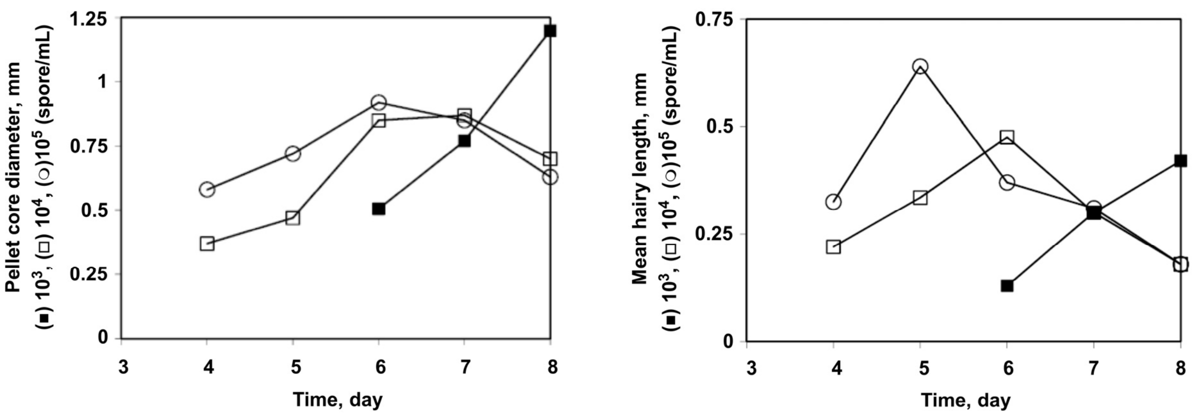


Figure 5: Representative pictures of clumps after: a) day 5 b) day 6 and c) day 7 of cultivation of *S. erythraea* (NUR001) for an initial spore concentration of 10^7 spore/mL



(a) **(b)**

Figure 6: Changes of: (a) pellet core diameter, and (b) mean pellet hairy length during *S. erythraea* cultivation for initial spore concentrations of 10^3, 10^4 and 10^5 spore/ml

The pellet mean hairy length initially increased and then sharply decreased as the *S. erythraea* cultivation advanced (Figure 6b). The maximum mean hairy length attained during *S. erythraea* cultivation increased with the increase in initial spore concentration. To the knowledge of the authors, there is no previous report on the variation of main hairy length during cultivation of actinomycetes, but this result is consistent with those reported for *Aspergillus terreus* (Rodríguez Porcel *et al*., 2005) and *Aspergillus awamori* (Cui *et al*., 1997) fermentations.

The pH of the fermentation media for the different runs was measured daily (results not presented) and varied in the range 5.5-7, depending on the cultivation time and initial spore concentration.

Comparison of the trends of change of dry cell weight and erythromycin production with cultivation time (Fig. 7a and b respectively) indicates that, irrespective of the type of morphology of the cells or the rheology of the cultivation broth, erythromycin production by *S. erythraea* is growth-associated. The production of secondary metabolites, such as antibiotics, is usually induced under nutrient limitation or other stress conditions. This usually, but not always, means that secondary metabolite production starts when the growth rate declines (i.e., antibiotic production is growth diossociated). In the case of erythromycin production by *S. erythraea,* previous research has shown that, depending on the environment of *S. erythraea* cultivation, erythromycin production can be both growth and non-growth associated. (Potvin and Péringer, 1994a, b; Bushell *et al*., 1997b; Clark *et al*., 1995; McDermott *et al*., 1993). For example, the use of ammonium sulfate (Potvin and Péringer, 1994a, b), chemostat cultivation under phosphate limitation (Trilli *et al*, 1987) and batch culture under N (nitrate) limitation (McDermott *et al*., 1993; Bushell *et al*., 1997b) has been reported to result in growth-associated erythromycin production. Various explanations have been given for this phenomenon: Potvin and Peringer (1994b) maintain that erythromycin production by *S. erythraea* under nutrient-rich conditions can be induced by local diffusional limitations imposed by early formation of mycelia pellets; on the other hand, Bushell *et al*. (1997b) claim that growth-associated erythromycin production can occur with growth substrates for which *S. erythraea* has low affinity. Since, in the present study, a complex media was employed for the cultivation of S. *erythraea,* no explanation can be given for the reason why erythromycin production was found to be growth-associated.

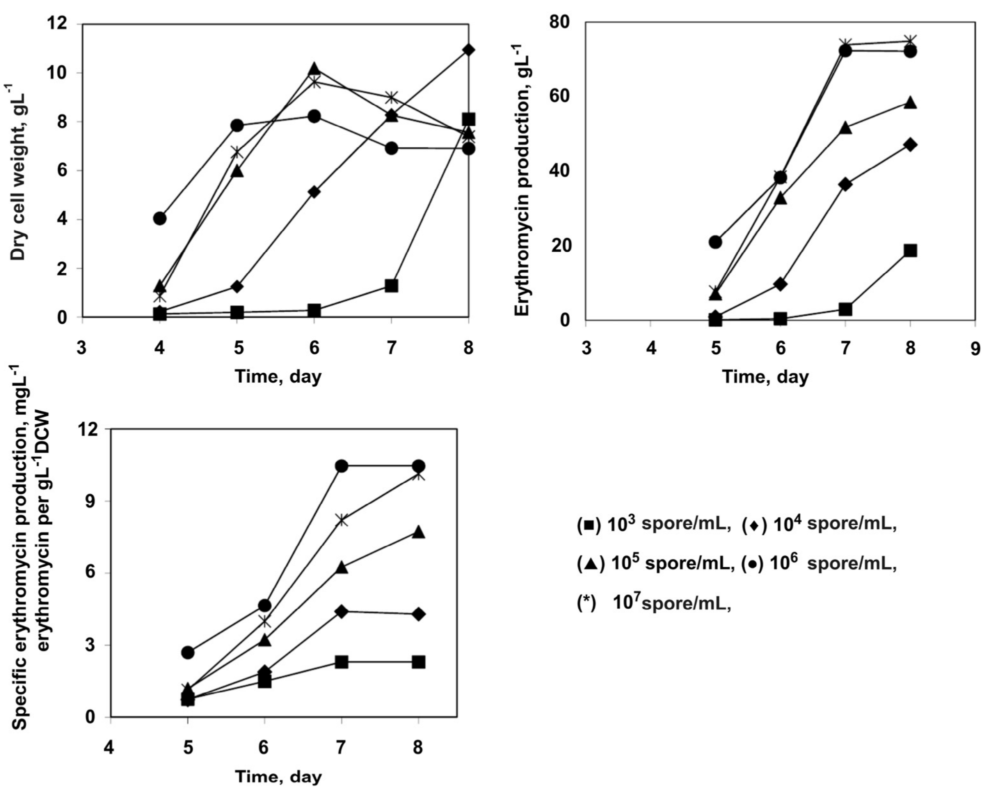


Figure 7: Changes in: (a) dry cell weight, (b) erythromycin production and (c) specific erythromycin production with cultivation time of S. *erythraea*

The data presented in Fig 7c show that specific erythromycin production is higher for runs at 10^6 and 10^7 spore/ml initial spore concentration compared to runs at lower initial spore concentrations. This correlates with the predominance of clump morphology in the former runs. Furthermore, in the range 10^3 to 10^5 spore/ml initial spore concentration, erythromycin production increases with an increase in the proportion of clump morphology. These two observations suggest that, for *S. erythraea*, clump morphology is more suitable for erythromycin production compared to pellet morphology. Oxygen limitation in the core of the pellet could be the main reason for lower erythromycin productivities for this type of morphology (Chen and Wilde, 1991). In the case of runs in which the clump morphology was noteworthy, the runs at an initial spore concentration of 10^6 spore/ml resulted in the highest erythromycin productivities; furthermore, the data show that this parameter consistently increases during *S. erythraea* cultivation. These two observations, together with the data presented in Figure 4 and Figure 3b, suggest that smaller clumps plus reduced non-Newtonian rheological characteristics of the cultivation broth improve erythromycin production. These observations might be related to oxygen limitations that occur with an increase in both clump dimension and *S. erythraea* cultivation broth viscosity. This can perhaps also explain why, at earlier cultivation times, the erythromycin productivity data (Figure 7c) were more similar to each other for runs at different initial spore concentrations. The oxygen limitation due to the high viscosity of the cultivation broth can be circumvented to a significant extent by carrying out the fermentation in well aerated, agitated bioreactors. However, the oxygen diffusion resistances due to the large dimension of the clumps consist of internal and external resistances and only the latter will be reduced if the fermentation is carried out in well mixed bioreactors.

CONCLUSIONS

The results obtained in the present work show that in submerged cultivation of *S. erythraea* a change in the initial spore concentration in the range 10^3 to 10^7 spore/mL had a determining effect on the morphology of the bacterial cells. The predominance of clump morphology increased and pellet morphology decreased with the increase in initial spore concentration. In runs in which there was sizeable pellet morphology, the rheology of the broth was Newtonian and pellet size was the largest and the mean pellet hairy length the lowest in runs at the lowest initial spore concentration. In the runs in which clump morphology was significant, the rheology of *S. erythraea* cultivation broths became more non-Newtonian with an increase in initial spore concentration. In these runs, a rough correlation was observed between the trend of change of morphological and rheological parameters. The mean major axis of the clumps was the lowest in runs with 10^6 initial spore concentration, which were the runs in which the highest erythromycin productivity was attained. Based on the results presented in the current study, it can be concluded that, for *Saccharopolyspora erythraea* cultivation, clump morphology is more suitable for erythromycin production compared to pellet morphology Furthermore, a decrease in clump dimensions, together with lower non-Newtonian broth viscosities, probably as a result of decrease in mass transfer resistances, also enhances erythromycin productivity.

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REFERENCES

Braun, S., Vecht-Lifshitz, S. E., Mycelial morphology and metabolite production. Trends in Biotechnology, 9, 63-68 (1991).

Bushell, M. E., Dunstan, G. L., Wilson, G. C., Effect of small scale culture vessel type on hyphal fragment size and erythromycin production in *Saccharopolyspora erythraea*. Biotechnology Letters, 19, 849-852 (1997a).

Bushell, M. E., Smith, J., Lynch, H. C., A physiological model for the control of erythromycin production in batch and cyclic fed batch culture. Microbiology, 143, 475-480 (1997b).

Chen, H. C., Wilde, F., The effect of dissolved oxygen and aeration rate on antibiotic production of *Streptomyces fradiae*. Biotechnology and Bioengineering, 37, 591-595 (1991).

Clark, G. J., Langley D., Bushell, M. E., Oxygen limitation can induce microbial secondary metabolite formation: investigations with miniature electrodes in shaker and bioreactor culture. Microbiology, 141, 663-669 (1995).

Cui, Y. Q., Van der Lans, R. G. J. M., Luyben, K. C. A. M., Effect of agitation intensities on fungal morphology of submerged fermentation. Biotechnology and Bioengineering, 55, 715-726 (1997).

Dobson, L. F., O'Cleirigh, C. C., O'Shea, D. G., The influence of morphology on geldanamycin production in submerged fermentations of *Streptomyces hygroscopicus* var. geldanus. Applied Microbiology and Biotechnology, 79, 859-866 (2008).

Du, L. X., Jia, S. J., Lu, F. P., Morphological changes of *Rhizopus chinesis* 12 in submerged culture and its relationship with antibiotic production. Process Biochemistry, 38, 1643-1646 (2003).

El-Enshasy, H. A., Farid, M. A., El-Sayed, E. S. A., Influence of inoculum type and cultivation conditions on natamycin production by *Streptomyces natalensis*. Journal of Basic Microbiology, 40, 333-342 (2000).

Glazebrook, M. A., Vining, L. C., Growth morphology of *Streptomyces akiyoshiensis* in submerged culture: influence of pH, inoculum and nutrients. Canadian Journal of Microbiology, 38, 98-103 (1992).

Hamedi, J., Malekzadeh, F., Niknam, V., Improved production of erythromycin by *Saccharopolyspora erythraea* by various plant oils. Biotechnology Letters, 24, 697-700 (2002).

Hamedi, J., Malekzadeh, F., Saghafi-nia, A. E., Enhancing of erythromycin production by *Saccharopolyspora erythraea* with common and uncommon oils. Journal of Industrial Microbiology and Biotechnology, 31, 447-456 (2004).

Heydarian, S. M., Mirjalili N., Ison, A. P., Effect of shear on morphology and erythromycin production in *Saccharopolyspora erythraea* fermentations. Bioprocess Engineering, 21, 31-39 (1999).

Kim, J., Hancock, I. C., Pellet forming and fragmentation in liquid culture of *Streptomyces griseus*. Biotechnology Letters, 22, 189-192 (2000).

Lawton, P., Whitaker, A., Odell, D., Stowell, J. D., Actinomycete morphology in shaken culture. Canadian Journal of Microbiology, 35, 881-888 (1989).

McDermott, J. F., Lethbridge, G., Bushell, M. E., Estimation of the kinetic constants and elucidation of trends in growth and erythromycin production in batch and continuous cultures of *Saccharopolyspora erythraea* using curve-fitting techniques. Enzyme Microbial Technology, 15, 657-663 (1993).

Papagianni, M., Mattey, M., Morphological development of *Aspergillus niger* in submerged citric acid fermentation as a function of the spore inoculum level. Application of neural network and cluster analysis for characterization of mycelial morphology. Microbial Cell Factories, 5:3. DOI:10.1186/1475-2859-5-3 (2006).

Potvin, J., Péringer, P., Ammonium regulation in *Saccharopolyspora erythraea*, Part II: Growth and antibiotic production. Biotechnology Letters, 16, 63-68 (1994a).

Potvin, J., Péringer, P., Ammonium regulation in *Saccharopolyspora erythraea*, Part II: Regulatory effects under different nutritional conditions. Biotechnology Letters, 16, 69-74 (1994b).

Rodríguez Porcel, E. M., Casas López, J. L., Sánchez Pérez, J. A., Fernández Sevilla, J. M., Chisti, Y., Effects of pellet morphology on broth rheology in fermentations of *Aspergillus terreus*. Biochemistry Engineering Journal, 26, 139-144 (2005).

Rostamza, M., Noohi, A., Hamedi, J., Enhancement in production of erythromycin by *Saccharopolyspora erythraea* by the use of suitable industrial seeding-media. DARU, 16, 13-17 (2008).

Sarra, M., Ison, A. P., Lilly, M., The relation between biomass concentration, determined by a capacitance-based probe, rheology and morphology of *S. erythraea*. Journal of Biotechnology, 51, 157-165 (1996).

Schatz, A., Waksman, S. A., Strain specificity and production of antibiotic substances. IV. Variation among actinomycetes, with special reference to *Actinomyces griseus*. Proceeding of the National Academy of Sciences, 31, 129-137 (1945).

Smith, C. M., Calam, C. T., Variations in inocula and their influence on the productivity of antibiotic fermentations. Biotechnology Letters, 2, 261-266 (1980).

Tresner, H. D., Hayes, J. A., Backus, E. J., Morphology of submerged growth of Streptomycetes as a taxonomic aid: I. morphological development of *Streptomyces aureofaciens* in agitated liquid media. Applied Microbiology, 15, 1185-1191 (1967).

Trilli, A., Crossley, M. V., Kontakou, M., The relationship between growth rate and erythromycin production in *Saccharopolyspora erythraea*. Biotechnology Letters, 9, 765-770 (1987).

Tucker, K. G., Thomas, C. R., Mycelial morphology: The effect of spore inoculum level. Biotechnology Letters, 14, 1071-1074 (1992).

Tucker, K. G., Thomas, C. R., Inoculum effects on fungal morphology: shake flasks vs. agitation bioreactors. Biotechnology Techniques, 8, 153-156 (1994).

Vecht-Lifshitz, S. E., Magdassi, S., Braun, S., Pellet formation and cellular aggregation of *Streptomyces tendae.* Biotechnology and Bioengineering, 35, 890-896 (1990).

Whitaker, A., Actinomycetes in submerged culture. Applied Biochemistry and Biotechnology, 32, 23-32 (1992).