Short communication

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Fermentation odor and bioprocess scale-up

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Abstract During scaleup of *Streptomyces avermitilis* batch culture based on similarity of geometry, aeration and mixing conditions, the production of avermectins was proportional to the intensity of fermentation odor represented by geosmin. The active stimulatory role of odor component on antibiotic production was detected after addition of volatile compounds separated from the fermentation liquor by steam distillation.

1
Introduction

Fermentation odors follow most cultivations of microorganisms. To be odorous, a component of the fermentation broth must be in a gaseous or volatile state. In food industry, hundreds of such compounds are produced in fermentations [1] and finally affect the flavor of products. Recently [2], we reported a list of volatile odorous compounds that are pursuing growth and avermectins production in a submerse cultivation of *Streptomyces avermitilis*. Having applied the common scaleup procedure [3, 4] we tried to determine the aeration and mixing conditions in fermentors of similar geometry and different volume to achive the same final production of avermectins. As shown in Fig. 1, the project failed and we have found that there was an unknown and significant scaleup parameter which was not taken into account. When we analyzed backward our experimental data we found a relatively weak correlation between aeration and production rate than could be expected [4]. Based on an idea of process operators which attributed the strength of "earthy smell" of fermentation gases to production of avermectins we went back to our study [2] in order to evaluate the unknown "dimension" of bioprocess scaleup.

2
Material and methods

Strain and cultivation. The mutant strain *Streptomyces avermitilis C-18* obtained by genetic improvement [5] of ATCC 1267 was used. The inoculum was cultivated for 24 h in a complex medium containing: glucose 0.5%; soybean meal 1.5% and yeast extract 0.5%; fermentation flasks were inoculated with 2% of the inoculum (24 h old) and cultivated for 10 days. For cultivation, the following synthetic production medium (%) was used: glucose 3, $(NH_4)_2SO_4$ 0.2, $CaCO_3$ 0.5, NaCl 0.2, K_2HPO_4 0.05, $FeSO_4 \cdot 7H_2O$ 0.005, $MnSO_4 \cdot 7H_2O$ 0.005, $ZnSO_4 \cdot 7H_2O$ 0.005, $MgSO_4 \cdot 7H_2O$ 0.01. In 500 ml round bottom flasks, the cultivation was performed on a rotary shaker (2.8 Hz) at 28 °C [5]. In mechanical stirred fermenter (1liter Bioflo, New Brunswick Sci.Co. N.J.,USA, 7 liter MBR BioReactor A.G, Swiss and 50 liter Bioengineering, Swiss) the cultivation was performed at same temperature, aeration rate 0.6 VVM and impeller tip speed 0.5–0.6 m/s. The 50 liter fermentor was equipped with efficient reflux condenser of volatile compounds inherent in outlet air. All fermentors were mixed by two similar six blade Rushton turbines and the vessels had four baffles.

Analytical methods. Content of total avermectins in a medium was detected by TLC [5]. Individual avermectins were determined by a reverse phase HPLC (Perkin Elmer series 3B Liquid Chromatograph) [6]. The volatile compounds were determined after steam distillation and extraction by capillary GC–MS (Jeol DX 303), as described previously [2].

3
Results and discussion

After analysis of volatile compounds content in frozen samples of fermentation broth we selected geosmin as a marker of "earthy odor" [7]. Figure 2 shows the relationship between geosmin and avermectins concentration in the cultivations depicted in Fig. 1. As shown in the figure, most of experimental data obtained in fermentors can be grouped lower around the curve which differs from the upper one which represents the flask cultivation. There is no doubt that flask and fermentors differ in the rate of air ventilation. Therefore, the concentrations of gaseous and volatile products of fermentation has to be higher in shaking flask and lower in fermentors due to higher stripping rate. In the production phase, the intensity of "earthy odor" represented by geosmin is proportional to the product concentration. It confirms the above mentioned empirical experience of fermentor operators. However, we have had to decide whether the content of geosmin is an attendant byproduct of avermectins formation or if it play an active regulatory role in antibiotic biosynthesis. In medical practice, there are same evidences that odors produced by different bacteria could strongly affect the physiological functions and health of patients [8, 9]. The volatile compounds could act in small concentration like pheromones [10, 11]. At first, we treated 1 liter of fermented and filtered broth from 7 liter MBR BioReactor

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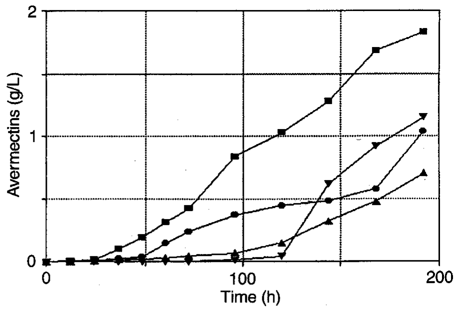

Fig. 1. Comparison of avermectins production in cultivation flasks (squares) and fermentors of different volumes (1lt fermentor – triangles bottom down, 7 lt fermentor – triangles bottom up and 50 lt fermentor – spheres)

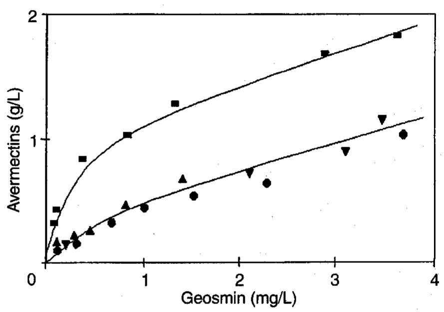

Fig. 2. Relationship between geosmin and avermectins concentrations

cultivation by steam distillation to obtain about 100 ml of water containing all volatile compounds [2]. Secondly, we filled ten cultivation flasks with 50 ml of synthetic production medium and inoculum. Next, we added 10 ml of steam distillate to five flasks and 10 ml of distilled water to other five flasks as a control. Afterward, the flasks were cultivated for 8 days and analyzed for the product. The control flasks contained 0.98 ± 0.07 and flasks filled with steam distillate had 1.5 ± 0.05 g avermectins/lt. The ratio between avermectins A and B was the same. The stimulatory effect of odor component on the production is apparent.

While there is a rich literature on bioreactor scaleup, the effect of volatile components of fermentation odor which may be very important has not been extensively explored. The components of odor spectrum could act in subtle concentrations like pheromones and thus to control the physiological response either microorganism itself or higher organisms, too. Probably, the low molecular odor compounds could react directly with DNA base sequence which serves as a receptor [12].

4
Conclusion

The design of efficient reflux condenser of a bioreactor is of same importance for bioprocess scale-up as optimal performance of an impeller, air sparger, baffles, geometry of bioreactor vessel, filters etc.

References

1. **Maarse, H.:** Volatile Compounds in Food and Beverages. N.Y.: M. Decker 1991
2. **Řezanka, T.; Líbalová, D.; Votruba , J.; Viden, I.:** Identification of odorous compounds from Streptomyces avermitilis. Biotechnol. Lett. 16 (1994) 75-78
3. **Singh, V.; Fuchs, R.; Hensler, W.; Constantinides, A.:** Scale-up and optimization of oxygen transfer in fermentors. Newtonian and Non-Newtonian systems. Ann. NY Acad. Sci. 589 (1990) 616-641
4. **Campbell, W. C.:** Ivermectin and abamectin. Berlin: Springer Verlag 1991
5. **Cimbůrková, E.; Zima, J.; Novák, J.; Vanìk, Z.:** Nitrogen regulation of avermectins biosynthesis in Streptomyces avermitilis in a chemically defined medium J.Basic Microbiol. 28 (1988) 491-499
6. **Čurdová, E.; Jechová, V.; Zima, J.; Vaněk Z.:** The effect of inorganic phosphate on the production of avermectin in Streptomyces avermitilis J.Basic Microbiol. 29 (1989) 341-346
7. **Řezanka, T.; Votruba, J.:** Effect of Salinity on Avermectines. Odour compounds and fatty acids formation by Streptomyces avermitilis. Folia Microbiol. 43 (1998) 47-50
8. **Lundholm, M.; Lavrell, G.; Mathiasson, L.:** Self - leveling mortar as a possible cause of symptoms assocaited with „sick building syndrome". Arch.Environ.Health.. 45 (1990) 135-40
9. **Neutra, R.; Lipscomb J.; Satin, K.; Shusterman, D.:** Hypotheses to explain the highes syptom rates observed around hazardous waste sites. Environ. Health Perspect. 94 (1991) 31–8
10. **Shepherd, G. M.:** Molecular vocabulary of olfaction. Ann N.Y. Acad. Sci. 510 (1987) 98-103
11. **Zalcman, S.; Kerr L.; Anisman, H.:** Immunosupression elicited by stressors and stressor-related odors. Brain Behav. Immun. 5 (1991) 262–73
12. **Ohno, S.:** Evolution by gene duplication. Berlin: Springer Verlag 1970