Scale-up of Recombinant Hirudin Production from *Saccharomyces cerevisiae*

Chul Ho Kim[1], K. Jagannadha Rao[1], Duk Joong Youn[2], and Sang Ki Rhee[1]*

[1] Metabolic Engineering Research Laboratory, Korea Research Institute of Bioscience and Biotechnology, 52, Oun-dong, Yusong, Daejeon 305-333, Korea
[2] Dong Kook Pharmaceutical Co. Ltd., P.O. Box 176, Youngdong, Seoul 135-283, Korea

Abstract Scale-up of hirudin production from *Saccharomyces cerevisiae* from bench-scale to pilot-scale was carried out based on constant volumetric oxygen transfer coefficient (K_La). Fed-batch mode of cultivation using step-wise feeding strategy of galactose was employed for the production of hirudin in a 30-L and a 300-L pilot-scale fermentor. The final hirudin concentrations were achieved 390 mg/L and 286.1 mg/L, and the volumetric productivities were 80.4% and 90.7% with the 30-L and 300-L fermentors, respectively, compared to the productivity of the 5-L bench-scale fermentor.

Keywords: recombinant hirudin, scale-up, fed batch fermentation, *Saccharomyces cerevisiae*, *GAL* promoter

Hirudin, a polypeptide of 65 amino acids, is a potent thrombin-specific inhibitor isolated from the salivary gland of the blood-sucking leech *Hirudo medicinalis* [1]. The limited availability of natural hirudin has encouraged the development of various fermentation processes, using recombinant microbial sources, for its large-scale production.

Efficient large-scale cultivation of recombinant *S. cerevisiae* for the production of a wide variety of products has been extensively reported [2]. Nevertheless, the complexity of the precise requirements for optimal growth of recombinant yeasts at high cell densities- maintaining high levels of expression of heterologous genes- mean that very little of the existing literature is applicable to the industrial scale [3]. The large-scale cultivation of *S. cerevisiae* at high cell densities must be carried out using a fed batch process. This type of culturing can eliminate problems of catabolic repression and allow parameters, such as oxygen consumption and heat production, that would otherwise be affected by reactor engineering constraints [4].

Effective scale-up is essential for successful bioprocessing. When a particular scale-up strategy is carried out by maintaining a specific set of parameters constant, other parameters can not be controlled and may change substantially in unexpected ways [5]. This may have undesired effects on the yield, because so many factors affect microbial growth and/or product formation. However, in aerobic fermentations oxygen is one of the most important substrates and a factor, that influences the direction of aerobic metabolism of microbial cells. Hence, a series of scale-up operations in aerobic cultures from a flask to bench-scale and from a bench-scale to a pilot- and industrial-scale, have been generally based on the volumetric oxygen transfer coefficient in order to ensure the same oxygen supply rate to support growth and metabolism of the desired high cell populations. Moreover, the suitability of scaled-up methods is usually confirmed by experimental results, which show that there is no difference in fermentation results between various types of small and large bioreators carried out under the same oxygen transfer rate coefficient. However, in practice, the results obtained with such scale-up methods often do not agree. This can be attributed to various factors. It has been reported that one of the causes for the disagreement is the differences in the effects of the hydrodynamic stress, generated by shaking operations and agitation in the reactors, on microbial cells themselves [6,7] and the geometric configurations of the reactors. Thus, for this reason, the scale-up of recombinant hirudin production from *S. cerevisiae* harbouring *GAL 10* promoter that developed in the previous study [8] was carried out with constant oxygen transfer rate coefficient held constant.

A step-wise feeding strategy of galactose that was developed in the previous study was employed for the fed-batch fermentations of hirudin [9]. The agitator speed was kept between 400-900 rpm to maintain the dissolved O_2 concentration at 20% air saturation level. Initially, fermentation was carried out like a batch experiment using a production medium, which consisted of yeast extract 40 g/L, casamino acids 5 g/L, glucose 20 g/L, KH_2PO_4 10 g/L. After 10 h of fermentation, glucose in the fermentation medium was exhausted and the dissolved O_2 concentration began to increase rapidly. At this

***Corresponding author**
Tel: + 82-42-860-4450 Fax: + 82-42-860-4594
e-mail: rheesk@kribb.re.kr

Table 1. Comparison of the final productivity of hirudin and cell mass obtained from bench-scale and pilot-scale fermentations by fed-batch fermentation with step-wise feeding of galactose

Fermentor type	Volumetric hirudin productivity ($\mu g\ L^{-1}\ h^{-1}$)	Cell growth (g/L)	Specific hirudin expression rate ($\mu g\ g\ cell^{-1}\ h^{-1}$)
Bench-scale			
5-L Jar Fermentor	6,840	90.7	75.4
Pilot-scale			
30-L Fermentor	5,500	70.1	78.5
300-L Fermentor	6,208	68.0	91.3

stage, galactose was fed into the fermentor by using step-wise feeding strategies, which was evaluated as a better feeding strategy in the previous study [9]. Galactose concentration was maintained at less than 40 g/L throughout fermentation using the feed solution containing galactose (500 g/L).

30-L and 300-L fermentors (Korea Fermentor Co.), with a working volume of 16 L and 160 L, respectively, were used for the scale-up studies. In the case of 300-L fermentor, the seed culture was developed in the 30-L fermentor and was inoculated at the 10% level of the 300-L fermentor. The K_La value in the fermentors was determined by the sulphite oxidation method. In the 30-L and 300-L scale, the agitation rates were varied between 160 to 200 rpm with aeration input of 0.6 to 0.8 vvm in order to maintain the constant oxygen transfer rate coefficient, K_La of 14.4/h. Other fermentation conditions, such as pH at 5.4 and temperature at 30°C, were maintained constant at all scales.

Scale-up of hirudin production from a 5-L jar fermentor to a 30-L and a 300-L pilot-scale fermentor has been investigated based on a constant oxygen transfer coefficient, K_La. Current scale-up methods assume that, as in the small-scale fermentor, the environmental conditions are homogeneously distributed within the large-scale fermentor. However, this is not true. There are so many factors, like hydrodynamic factors, height and geometric configuration of the reactor, that would affect the environment of the fluid in the large-scale reactors.

The supply of oxygen is the most important factor in the scale-up of submerged fermentations. Koizumi *et al.* [10] suggest that the possible damage from the high rates of aeration that correspond to the high oxygen transfer rate (OTR) values is counter-balanced by damage to the organisms in the highly turbulent fermentation broth. Consequently, most of the work on process optimization focused on the effects of aeration and agitation, while the volumetric oxygen transfer coefficient (K_La) and power consumption per unit liquid volume (P/V) are widely used as scale-up parameters. Many processes were successfully scaled-up on the basis of constant K_La and P/V. Scale-up based on constant P/V is usually adopted for larger-scale fermentors, such as those above 1,000-L capacity. In this investigation, scale-up of hirudin production from a 5-L jar fermentor to a 300-L pilot-scale fermentor was carried out using constant K_La (14.4/h) as a scale-up parameter. As shown in Table 1 and Figs. 1 and 2, the volumetric hirudin productivities in the 30-L and 300-L fermentors were 80.4% and 90.7%, respectively, in comparison with the 5-L bench-scale fermentor. However, the specific expression rate of hirudin was increased in both the 30-L and 300-L fermentors compared to the 5-L jar fermentor. In the 300-L fermentor, the specific expression rate of hirudin increased by 1.21 fold the rate obtained with the 5-L jar fermentor. The cell growth decreased in both of the 30-L and 300-L fermentors. This could be due to the hydrodynamic stress that the cells underwent at the higher-scale fermentors. These results suggest that the scale-up of recombinant hirudin production on the basis of constant K_La alone was not a favorable method, which indicates that there are some other limiting factors other than oxygen supply that affects the hirudin production in higher-scale fermentors.

The major differences between small and large fermentors include: a) physical factors, such as impeller diame-

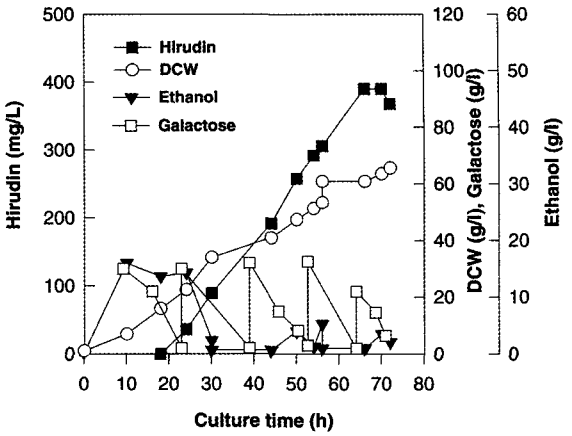

Fig. 1. Fed-batch fermentation profiles of r-hirudin with step-wise feeding of galactose in 30-L fermentor.

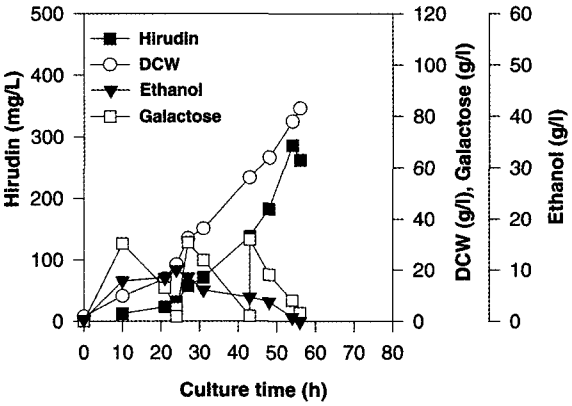

Fig. 2. Fed-batch fermentation profiles of r-hirudin with step-wise feeding of galactose in 300-L fermentor.

ter, which result in differences in the shear stress exerted on the cells, and b) factors relating to the high liquid levels in the large fermentors such as the differences in liquid pressure, dissolved oxygen and carbon dioxide concentrations. These factors are currently under investigation and we are also considering other ways for successful scale-up, in other words, at 100% productivity of the laboratory scale.

In conclusion, step-wise feeding of galactose was found to be a promising feeding strategy and is recommended on the basis of operational and production-yield aspects. Scale-up based on constant K_La produced volummetric productivity of r-hirudin at 300-L pilot-scale that was 96.8% that of the 5-L bench-scale.

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