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ORIGINAL PAPER

Kinetic studies of recombinant human interferon-gamma expression in continuous cultures of *E. coli*

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Abstract A series of continuous cultures was performed to understand the product formation kinetics of recombinant human interferon gamma (rhIFN-γ) in *Escherichia coli* at different dilution rates ranging from 0.1 to 0.3 h^{-1} in different media. A T7 promoter-based vector was used for expression of IFN-γ in *E. coli* BL21 (DE3) cells. The recombinant protein was produced as inclusion bodies, thus allowing a rapid buildup of rhIFN-γ inside the cell, with the specific product yield ($Y_{p/X}$) reaching a maximum value of 182 mg g^{-1} dry cell weight (DCW). In all the media tested, the specific product formation rate (q_p) was found to be strongly correlated with the specific growth rate (μ), demonstrating the growth-associated nature of product formation. The q_p values show no significant decline with time postinduction, even though the recombinant protein has been over produced inside the cell. The maximum q_p level of 75.5 mg g^{-1} h^{-1} was achieved at the first hour of induction at the dilution rate of 0.3 h^{-1}. Also, this correlation between q_p and μ was not critically dependent on media composition, which would made it possible to grow cells in defined media in the growth phase and then push up the specific growth rate just before induction by pulse addition of glucose and yeast extract. This would ensure the twin objectives of high biomass and high specific productivities, leading to high volumetric product concentration.

Keywords Recombinant human Interferon gamma · *E. coli.* kinetics · Continuous culture

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Introduction

The complex nature of cellular reactions has been a major obstacle in understanding the kinetics of product formation in recombinant cultures. Previous authors have modeled the expression of intracellular recombinant proteins as a function of the specific growth rate (μ) both pre- and postinduction and the changes in cellular physiology, which influence the carbon supply for biomass and product synthesis [1, 2]. It has been reported that recombinant protein expression increases with the increase in preinduction μ [3, 4]; however, there had been contradictory reports showing a decline in the concentration of recombinant product when μ was increased [2, 5]. On the other hand, there are few reports that demonstrate the absence of any correlation between these two factors [6–8]. Some authors have claimed that rather than the preinduction μ, it is a high postinduction μ, which helps in achieving high-level production of recombinant protein [9], whereas others have shown that the postinduction μ is an intrinsic property of the cell that cannot be controlled by bioprocess strategies [10, 11]. However, one problem associated with some of these reports is that they have looked at overall expression levels rather than the specific product formation rate (q_p), which is a better measure of product formation kinetics. The other more important reason behind this observed variation in the relationship between expression levels and μ is because different bottlenecks such as toxicity, codon

bias, etc., govern the expression of different recombinant proteins.

The specific productivity may depend on many factors, such as higher ribosome content at higher μ [6, 12], low accumulation of byproducts, availability of continuous nutrient supply, and plasmid stability [13, 14]. Thus, arguing conversely an elucidation of the product formation kinetics in recombinant cultures would be a useful bioprocess tool in the identification of the primary bottleneck to recombinant protein expression.

In this study, recombinant human interferon gamma (rhIFN-γ) was chosen as a model system for the following reasons. First, the high expression level of rhIFN-γ in *Escherichia coli* provides an interesting system for kinetic modeling where a large metabolic flux is diverted for product formation [15]. Second, it has potential therapeutic uses in kidney-cell carcinoma, colon cancer, and rheumatoid arthritis [16]. Third, rhIFN-γ produced in *E. coli* is not glycosylated and has a molecular weight of 17 kDa but is still active physiologically [17].

Escherichia coli is one of the most widely used hosts for the production of heterologous proteins [18]. Most recombinant proteins expressed in *E. coli* accumulate intracellularly and hence volumetric productivity are proportional not only to the final cell density but also to the specific product yield ($Y_{p/X}$). The main objective of this study was to examine the synergy between the effect of μ and media composition on the kinetics of product formation while expressing the rhIFN-γ under a strong promoter. The study was carried out using continuous cultures of *E. coli* by selecting three commonly used media: namely, Terrific broth (TB), Luria broth (LB), and minimal media containing 0.2% yeast extract (M9YE). The correlation between μ and q_p was determined by conducting the continuous culture studies at different dilution rates.

Materials and methods

Host strain and expression vectors

Escherichia coli BL21 (DE3) was obtained from Stratagene (USA) and used for expression studies. The T7-based expression vector pET14b (Novagen), having an ampicillin resistance marker, was used for cloning the rhIFN-γ gene. Two oligonucleotide primers 5′-TTG GCT GTC ATA TGC AG GAC CCA TAT GTA-3′ and 5′-GGA GGA TCC TTA CTG GGA TGC TCT-3′ (Microsynth, Switzerland) bearing the *Nde1* and *BamH1* restriction sites, respectively, were used to amplify the rhIFN-γ complementary DNA (cDNA). The AUG codon needed for translational initiation was automatically introduced through the *Nde1* site. Peripheral blood mononuclear cells (PBMCs) from a single blood donor was isolated and cultured in Roswell Park Memorial Institute (RPMI) 1640 with 10% fetal calf serum and 10 mg ml^{-1} *Mycobacterium tuberculosis* (H37RV) antigen to induce IFN-γ expression. Total RNA was isolated from harvested cells after 48 h (High Pure RNA Isolation Kit, Roche), which was used to amplify the rhIFN-γ cDNA by reverse transcriptase polymerase chain reaction (RT-PCR) (C-therm Polymerase One Step RT-PCR System, Roche). The amplified cDNA fragment was cloned between the *Nde1* and *BamH1* sites of plasmid pET14b (Novagen) to obtain the plasmid pET14-IFN-γ.

Growth media

Three different media were used for the cultivation of bacterial cells viz. LB (10 $g1^{-1}$ bacto tryptone, 5 g l^{-1} yeast extract, 5 g l^{-1} NaCl); M9YE (0.5 g l^{-1} NaCl, 1 g l^{-1} NH_4Cl, 3 g l^{-1} K_2HPO_4, 0.1 ml l^{-1} 1 M $CaCl_2$, 2 ml l^{-1} 1 M $MgSO_4$, 0.2% glucose and 0.2% yeast extract), and TB (24 g l^{-1} yeast extract, 12 g l^{-1} tryptone, 0.4% glycerol, 2.31 g l^{-1} KH_2PO_4 and 12.54 g l^{-1} K_2HPO_4). All media were supplemented with 150 μg ml^{-1} (1.5×) ampicillin throughout the study.

Culture conditions

Shake-flask studies were done in 500-ml conical flasks for 100-ml cultures with constant agitation at 200 rpm at 37°C. Bioreactor studies were performed in a 2-L bioreactor (Bio-Flo 2000, New Brunswick Scientific, NJ, USA) with a working volume of 1 l under the following conditions: aeration, 1 vvm; temperature, 37°C; pH maintained at 7.2 automatically with acid and base pumps; dissolved oxygen maintained at 40%. Single colonies were inoculated in 10 ml of growth medium containing 100 μg ml^{-1} ampicillin. Cells were grown overnight at 37°C with constant shaking at 200 rpm. This primary culture was inoculated into 100-ml medium in a 500-ml conical flask and cells grown under the same conditions. This cell culture at its mid log phase was used as an inoculum in the fermentor containing 900 ml medium and ampicillin (1×). The inlet and outlet pumps attached to the fermentor were switched on when the culture reached appropriate cell density in order to get the desired dilution rate of the continuous culture.

Biomass measurement

Biomass was measured by dry cell weight (DCW L^{-1}) and optical density methods, as reported previously [19]. The cell pellet obtained in a preweighed centrifuge after spinning 1 ml of the cell culture was washed with deionized water and spun again at 10,000×g for 5 min. The

supernatant was discarded properly, and the cells were dried at 80°C to get DCW. The optical density (OD) of the culture after appropriate dilution was measured by Hitachi model U2000 spectrophotometer at 600 nm. A correlation was derived between DCW and OD_{600}, which showed that one OD_{600} corresponded to 0.4 g l^{-1}(DCW).

Plasmid stability

Serially diluted samples from the bioreactor were spread on LB agar plates without ampicillin. The total number of colonies that appeared after overnight incubation at 37°C was counted as total viable cells. These colonies (~100) were further transferred to LB ampicillin plates and incubated overnight at 37°C. The percentage plasmid stability was calculated from the number of colonies that were able to grow on ampicillin plates.

ELISA

Enzyme-linked immunosorbent assay (ELISA) kit (Prepotech, USA) was used to detect and quantify the amount of rhIFN-γ from the cell samples as per the manufacturer's instructions. The total rhIFN-γ was quantified using a standard curve for rhIFN-γ, with the samples being diluted appropriately so that they remained in the linear range of ELISA reading. Samples for ELISA were prepared by resuspending the cell pellets in 2% sodium dodecyl sulfate (SDS) and boiled for 1 min prior to serial dilution, as reported earlier [19].

Results

Cloning of the human IFN-γ gene

Human IFN-γ cDNA was synthesized by RT-PCR (as mentioned in "Materials and methods") and the amplified fragment rhIFN-γ was cloned into pET14b in between the *BamH1* and *Nde1* restriction sites under the control of T7 promoter. The resulting plasmid pET14-IFN-γ was used to transform *E. coli* BL21 (DE3) cells, and the expression of rhIFN-γ was checked by SDS-PAGE and ELISA. Sequencing of the gene was done for confirmation, and the gene sequence was submitted to GenBank bearing the accession number AY044154.

Shake-flask studies

At the shake-flask level, rhIFN-γ expression was determined by transforming *E. coli* BL-21(DE3) cells with plasmid pET14-IFN-γ. Transformed cells were grown in LB, M9YE, and TB medium and induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) when the cells reached mid-log phase. The amount of rhIFN-γ produced was quantified by ELISA, and the maximum $Y_{p/X}$ found to be 140, 130, and 115 mg g^{-1} DCW for TB, LB, and M9YE, respectively (data not shown). However, TB being a highly enriched medium, the final OD_{600} reached 8.5; hence, the volumetric product concentration also exceeded the other two media by >3.3 fold. All the media showed different responses in terms of productivity and growth rate; hence, it was important to delink the effect of media composition and the specific growth rate on the product formation kinetics. Therefore, continuous culture studies were performed in TB, LB, and M9YE at different dilution rates of 0.1, 0.2, and 0.3 h^{-1}.

Continuous culture studies at 0.3 h^{-1}

Batch cultures were conducted in the three media by growing the cells in a 1-L bioreactor, and feed was started at the rate of 300 ml h^{-1} to achieve a desired dilution rate of 0.3 h^{-1}. The culture was induced by 1 mM IPTG when the system reached a steady state (i.e., OD_{600} becomes constant) and continued for 6 h postinduction (Fig. 1).

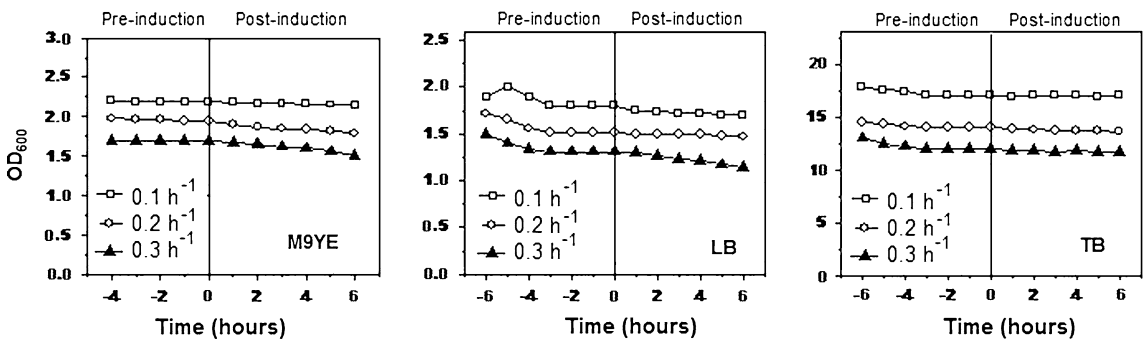

Fig. 1 Continuous cultures of *Escherichia coli* BL-21(DE3) cells for expression of recombinant human interferon gamma (rhIFN-γ) in separate media under different dilution rates. The *vertical line* indicates the point of induction for each experiment. Each data point is the average value derived from two different experiments, and the maximum deviation was <10% in every case

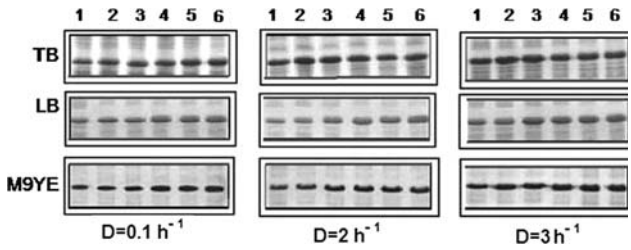

Fig. 2 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of recombinant human interferon gamma (rhIFN-γ) expression using total cell lysates from continuous cultures at dilution rates of 0.1, 0.2, and 0.3 h^{-1} using different media. *Lanes 1 to 6* indicate level of rhIFN-γ expression from 1 h to 6 h postinduction period, respectively

Samples were collected every hour for product analysis using SDS polyacrylamide gel electrophoresis (PAGE) and ELISA. In TB medium, there was no washout, indicating that the postinduction μ was the same as before induction; however, there was a very slow washout in case of LB and M9YE (Fig. 1). SDS-PAGE was run to analyze the production of rhIFN-γ in different media, as shown in Fig. 2. In TB medium, $Y_{p/X}$ value reached 175 mg g^{-1} DCW after 4 h postinduction and finally reached a maximum of 182 mg g^{-1} DCW after 6 h, as shown in Table 1. At this point, rhIFN-γ comprised $\sim$35% of the total cellular protein comparatively close to LB (32%) and M9YE (30%). However, the maximum volumetric concentration in TB was 0.85 g l^{-1}, which is around 10-fold higher compared with LB and M9YE. The plasmid stability was checked for all the above experiments and found to be 100% stable throughout the cultivation (data not shown).

Table 1 Results showing the maximum values of specific product formation rate (q_p), specific product yield ($Y_{p/X}$), and biomass obtained from continuous culture experiments under different conditions

Media	Dilution rates (h^{-1})	q_p (mg $g^{-1}h^{-1}$)	$Y_{p/X}$ (mg g^{-1} DCW)	Biomass (g 1^{-1})
TB	0.1	23.5	89.0	6.8
	0.2	53.0	150.0	5.6
	0.3	75.5	182.0	4.8
LB	0.1	23.0	88.0	0.68
	0.2	51.0	142.0	0.60
	0.3	68.0	170.0	0.52
M9YE	0.1	22.5	85.0	0.85
	0.2	46.0	115.0	0.76
	0.3	75.5	160.0	0.66

All data are represented as mean values of two independent analyses with <10% standard error

TB Terrific broth, *LB* Luria broth, *M9YE* minimal media containing 0.2% yeast extract

Continuous culture at 0.2 and 0.1 h^{-1}

Similar runs were performed at the desired dilution rates. In all the media tested, the $Y_{p/X}$ value was 1.2–1.6 times lower when dilution rate was reduced to 0.2 and 0.1 h^{-1}, as shown in Table 1. As observed earlier, there was no washout at these dilution rates in any of the media (Fig. 1). Production level was also estimated by SDS-PAGE in different media at different dilution rates (Fig. 2).

Discussion

The rational design of a feed strategy for overexpressing a desired protein is critically dependent on the product formation kinetics, as it is important to maintain a high specific productivity while increasing the biomass concentration. Otherwise, the gains of high-cell-density cultivation would be offset by low per-cell productivity, and product concentrations will not increase proportionally with the biomass concentration. As both TB and LB gave similar product formation kinetics, it was decided to use defined medium to check the expression of rhIFN-γ. For this, rhIFN-γ was cloned into pET14b in between the *BamH1* and *Nde1* restriction sites. However, we observed extremely poor growth in M9 media, and therefore it was decided to supplement this media with 0.2% yeast extract to provide essential nutrients for growth.

Interestingly, there was hardly any drop in the specific growth rate postinduction, as has been reported by previous authors [20, 21]. The biomass remained constant for all dilution rates, even though at a dilution rate of 0.3 h^{-1} there was a very slight decline in OD at 600 nm in LB and M9YE (Fig. 1). This marginal decline in OD shows the ability of the recombinants to effectively compete with the emergence of nonrecombinants in the culture, which was primary reason for getting 100% stability. The fall in specific growth rate postinduction has been reported primarily due to the metabolic stress associated with recombinant protein expression [22]. This stress depends on the nature and toxicity of the foreign protein toward the host. Thus, expression of a toxic protein tends to reduce or even stop the growth of the host cell [23–25]. However, in our case, rhIFN-γ expression resulted in formation of inclusion bodies, which sequester the protein inside the cell, thereby the stress related to the cytoplasmic presence of the protein is usually negligible. Thus, the nature of stress with inclusion body formation [26] is quite different from the situation when a soluble protein is overexpressed [27]. Also, it is important to note that the reduction in specific growth rate depends primarily on the nonavailability of a few critical metabolites, which can be supplemented by an exogenous supply of these metabolites. The presence of an

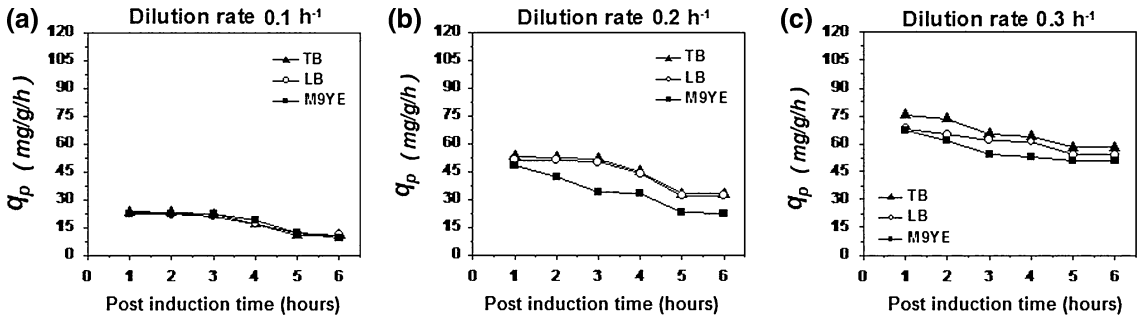

Fig. 3 Specific product formation rate (q_p) versus postinduction time (h) in different media at various dilution rates of **a** 0.1 h^{-1}, **b** 0.2 h^{-1}, and **c** 0.3 h^{-1}. Each point is the average of two duplicate tests, and the maximum deviation is <10%

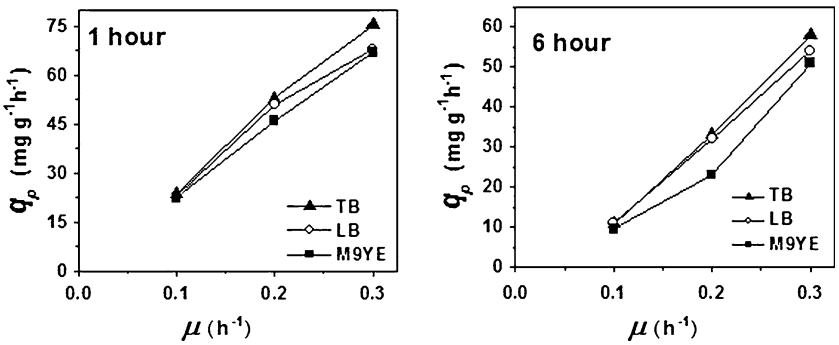

Fig. 4 Specific product formation rate (q_p) versus specific growth rate (μ) in different media at 1 h and 6 h postinduction period. Data is the average of two analyses, and the maximum standard deviation of the duplicates did not exceed 10%

exogenous supply of amino acids could be a critical factor in recombinant protein expression [28, 29]. This could be one reason we observed a declined in μ when we used M9YE medium in comparison to rich media such as TB where the availability of an exogenous amino acid pool helps largely in overcoming the metabolic stress associated with recombinant protein expression. However, the OD_{600} levels obtained at higher dilution rates were significantly lower, demonstrating that the residual substrate concentrations were quite high.

We also made an attempt to calculate the maintenance coefficient by looking at the change in the biomass yield coefficient postinduction. The oxygen yield and the respiratory quotient were calculated by measuring the oxygen and carbon dioxide fraction in the exit gas, as complex media precluded precise measurements of substrate uptake rates. However, we observed that the maintenance coefficient values were not high enough to be estimated by this technique, no there was significant change within experimental error of $Y_{x/o}$ (data not shown).

The specific product formation rate $\left(q_p = \frac{1}{X}\frac{dP}{dt}\right)$ was calculated by including the contribution of the increase in product concentration per unit cell, as well as the outflow of the product from the bioreactor (the dilution effect):

$$\frac{d(p.X)}{dt} = q_p X - DpX$$

where 'X' is the biomass concentration; 'p' is product concentration per unit biomass, and 'D' is dilution rate (at steady state $D = \mu$). As 'X' was constant in almost all cases postinduction (no washout), it could be taken outside the derivative term. Also, as the product was intracellular, the product concentration 'p' was the same as the specific product yield ($Y_{p/X}$). Thus,

$$q_p = \frac{d(Y_{p/X})}{dt} + DY_{p/X}$$

This equation was used to calculate the q_p values postinduction and q_p was also plotted against time for all dilution rates in different media, as shown in Fig. 3. There was no significant change in the q_p values postinduction, which further supports our earlier presumption that the metabolic stress associated with recombinant protein synthesis has no impact in our case. It is also clear that q_p values were significantly higher throughout the postinduction period when higher dilution rates were used, and the maximum values of $Y_{p/X}$ and of q_p were achieved at higher dilution rates in all the media (Table 1). A strong relationship could be thus established between q_p and μ. A plot of q_p versus μ at 1 h and 6 h postinduction clearly demonstrates the growth-associated nature of product formation (Fig. 4). Thus, this growth-associated nature of product formation could preferably lead to higher q_p while maintaining a high specific growth rate (μ) at the time of induction. Even though we observed that q_p was strongly correlated with μ, it could also be affected by other culture parameters, such as media composition. Interestingly, however, the correlations obtained between

q_p and μ were not critically dependent on media composition, as is obvious from the similar q_p versus μ plots taken at different time points. Therefore, it would be possible to grow cells in defined media in the growth phase and then push up the specific growth rate just before induction by pulse addition of glucose and yeast extract. This would ensure the twin objectives of high biomass and high specific productivities leading to high volumetric product concentration. Besides, this study could also be useful in designing a strategic methodology for overproduction of recombinant proteins in the form of inclusion bodies and possible diversion of maximum metabolic flux toward target protein synthesis.

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References

1. Stephanopoulos G, Vallino JJ (1991) Network rigidity and metabolic engineering in metabolite overproduction. Science 252:1675–1681
2. Turner C, Gregory ME, Turner MK (1994) A study of the ffect of specific growth-rate and acetate on recombinant protein-production of *Escherichia coli* JM107. Biotechnol Lett 16:891–896
3. Siegel R, Ryu DDY (1985) Kinetic study instability of recombinant plasmid pPLc23trp in *E. coli* using two-stage continuous culture system. Biotechnol Bioeng 27:28–33
4. Jung G, Denefle P, Becquart J, Mayaux JF (1988) High-cell density fermentation studies of recombinant *Escherichia coli* strains expressing human interleukin-1 beta. Ann Inst Pasteur Microbiol 139:129–146
5. Seo JH, Baily JE (1986) Continuous cultivation of recombinant *E. coli*: existence of an optimum dilution rate for maximum plasmid and gene product concentration. Biotechnol Bioeng 28:1590–1594
6. Shin CS, Hong MS, Bae CS, Lee J (1997) Enhanced production of human mini-proinsulin in fed-batch cultures at high cell density of *Escherichia coli* BL21 (DE3) [pET-3aT2M2]. Biotechnol Prog 13:249–257
7. Bech Jensen E, Carlsen S (1990) Production of recombinant human growth hormone in *Escherichia coli*, Expression of different precursors and physiological effects of glucose, acetate, and salts. Biotechnol, Bioeng 36:1–11
8. Zabriskie DW, Arcuri EJ (1986) Factors influencing productivity of fermentations employing recombinant microorganism. Enzyme Microb Technol 8(December):706–717
9. Lim HK, Jung KH (1998) Improvement of heterologous protein productivity by controlling postinduction specific growth rate in recombinant *Escherichia coli* under control of the PL promoter. Biotechnol Prog 14:548–553
10. Panda AK, Khan RH, Mishra S, Appa Rao KBC, Totey SM (1999) Kinetic of inclusion body production in batch and high cell density fed-batch culture of *Escherichia coli* expressing ovine growth hormone. J Biotechnol 75:161–172
11. Ramalingam S, Gautam P, Mukherjee KJ, Jayaraman G (2007) Effects of post-induction feed strategies on secretory production of recombinant streptokinase in *Escherichia coli*. Biochem Eng J 33:34–41
12. Hoffman F, Heuvel JVD, Zidek N, Rinas U (2004) Minimizing inclusion body formation during recombinant protein production in *Escherichia coli* at bench and pilot plant scale. Enzyme Microb Technol 34:235–241
13. Hoffmann F, Rinas U (2004) Stress Induced by Recombinant Protein Production in *Escherichia coli*. Adv Biochem Engg/ Biotechnol 89:73–92
14. Lee SY (1996) High cell-density culture of *Escherichia coli*. Tibtech 14:98–105
15. Babaeipour V, Shojaosadati SA, Robatjazi SM, Khalilzadeh R, Maghsoudi N (2007) Over-production of human interferon-g by HCDC of recombinant *Escherichia coli*. Process Biochem 42:112–117
16. Zhang Z, Tong K-T, Belew M, Petterson T, Janson JC (1992) Production, purification and characterization of recombinant human interferon-γ. J Chromatogr 604:143–155
17. Khalilzadeh R, Shojaosadati SA, Maghsoudi N, Mohammadian-Mosaabadi J, Mohammadi MR, Bahrami A, Maleksabet N, Nassiri-Khalilli MA, Ebrahimi E, Naderimanesh H (2004) Process development for production of recombinant human interferon-γ expressed in *Escherichia coli*. J Ind Microbiol Biotechnol 31:63–69
18. Shiloach J, Kaufman J, Guillard AS, Fass R (1996) Effect of glucose supply strategy on acetate accumulation, growth, and recombinant *E. coli* BL21(λDE3) and *Escherichia col*i JM109. Biotechnol Bioeng 49:421–428
19. Srivastava P, Bhattacharya P, Pandey G, Mukherjee KJ (2005) Overexpression and purification of recombinant human interferon-alpha2b in *Escherichia*. Protein Expr Purif 41:313–322
20. Curless C, Pope J, Tsai L (1990) Effect of preinduction specific growth rate on recombinant alpha consensus interferon synthesis in *Escherichia coli*. Biotechnol Prog 6:149–152
21. Babu KR, Swaminathan S, Marten S, Khanna N, Rinas U (2000) Production of interferon-α in high cell density cultures of recombinant *Escherichia coli* and its single step purification from refolded inclusion body proteins. Appl Microbiol Biotechnol 53:655–660
22. Neubauer P, Lin HY, Mathiszik B (2003) Metabolic load of recombinant protein production: inhibition of cellular capacities for glucose uptake and respiration after induction of a heterologous gene in *Escherichia coli*. Biotechnol Bioeng 83:53–64
23. Otto CM, Niagro F, Su X, Rawlings CA (1995) Expression recombinant feline tumor necrosis factor is toxic to *Escherichia coli*. Clin Diagn Lab Immunol 2:740–746
24. Asoh S, Nishimaki K, Nanbu-Wakao R, Ohta S (1998) A trace amount of the human pro-apoptotic factor Bax induces bacterial death accompanied by damage of DNA. J Biol Chem 273:11384–11391
25. Wu CM, Chang MD (2004) Signal peptide of eosinophil cationic protein is toxic to cells lacking signal peptide peptidase. Biochem Biophys Res Commun 2:585–592
26. Jurgen B, Lin HY, Riemschneider S, Scharf C, Neubauer P, Schmid R, Hecker M, Schweder T (2000) Monitoring of genes that respond to overproduction of an insoluble recombinant protein in *Escherichia coli* glucose-limited fed-batch fermentations. Biotechnol Bioeng 70:217–224
27. Schweder T, Lin HY, Jurgen B, Breitenstein A, Riemschneider S, Khalameyzer V, Gupta A, Buttner K, Neubauer P (2002) Role of the general stress response during strong overexpression of a heterologous gene in *Escherichia coli*. Appl Microbiol Biotechnol 58:330–337
28. Yee L, Blanch HW (1993) Defined media optimization for growth of recombinant *Escherichia coli* X90. Biotechnol Bioeng 41:221–230
29. IIanes A, Acevedo F, Gentina JC, Reyes I, Torres R, Cartagena O, Ruiz A, Vasquez M (1994) Production of penicillin acylase from *Bacillus megaterium* in complex and defined media. Process Biochem 29:263–270