Evaluation of scale-up parameters of bioethanol production from Escherichia coli KO11

[Escherichia coli KO11 suşundan biyoetanol üretimi için ölçek büyümeye parametrelerinin değerlendirilmesi]

Irem Deniz¹,², Esra Imamoglu¹, Fazilet Vardar Sukan¹

¹Ege University, Department of Bioengineering, Faculty of Engineering, Izmir
²Celal Bayar University, Department of Bioengineering, Faculty of Engineering, Manisa

ABSTRACT

Objective: In recent years, increased attention has been devoted to the conversion of biomass into fuel ethanol, as one of the cleanest liquid fuel alternatives to fossil fuels. However, industrial production of bioethanol is related with successful scaling-up studies.

Methods: In this study, the experimental designs of scale-up procedures based on constant mixing time, impeller tip speed and oxygen mass transfer coefficient were performed in 8 L stirred tank reactor and were compared in terms of product yield and productivity with those obtained from 2 L stirred tank reactor using quince pomace as a substrate for bioethanol production by Escherichia coli KO11.

Results: Scale-up based on constant mixing time yielded a maximum ethanol concentration of 23.42 g/L which corresponded to 0.4 g ethanol/g reduced sugar in 8 L stirred tank reactor. Moreover, shear stress increased only 1.1 fold which resulted in low cell damage and high cell viability.

Conclusion: Constant mixing time was identified as the most important key parameter especially for scaling-up of viscous fermentation broths of bioethanol production due to the significance of the homogeneity.

Key Words: Bioethanol production, Escherichia coli KO11, mixing time, quince pomace, scale-up

Conflict of Interest: The authors have no conflict of interest.

ÖZET

Amaç: Son yıllarda fosil yakıtlara alternatiflerden biri olarak biyokütlenin dönüşümüyle etanol elde edisi çok önem kazanmıştır. Endüstriyel ölçek etanol üretim, başarılı bir ölçek büyümeye bağlıdır.

Metod: Bu çalışmada, 8 L karıştırmalı tank reaktörde sabit karışma süresi, sabit bıçak hızı ve sabit oksijen kütle transfer katsayısına göre ölçek büyümeye prosedürleri gerçekleştirilmiştir. Sonuçlar, Escherichia coli KO11 suşundan ayva posası ile 2 L reaktörde elde edilen sonuçlarla ürün verimi ve verimliliği bakımından karşılaştırılmıştır.

Bulgular: 8 L karıştırmalı tank reaktörde maksimum etanol konsantrasyonu (23.42 g/L), 0.4 g etanol/g indirgenmiş şeker verimli sabit karışma süresine göre yaptığımız ölçek büyümeye elde edilmiştir. Ayrıca, yüksek hücre canlılığına ve düşük hücre canlanması sonucunda hücre hasarını önlenmiştir.

Sonuç: Sabit karışma süresi, bioethanol üretiminde homojenitenin önemli açıdan özellikle viskoz fermentasyon sıvıları için önemlidir. Ölçek büyümeye analiz edilmiştir.

Çıkar Çatışması: Yazarların çıkar çatışması yoktur.

Conflict of Interest: The authors have no conflict of interest.

Conflict of Interest: The authors have no conflict of interest.
Introduction

During the last two decades, the conversion of agro-industrial wastes to ethanol has been intensively studied using genetically engineered Escherichia coli KO11. Recombinant E. coli KO11 has high ethanol yields, a high phenotypic stability and is capable of efficiently producing ethanol from all sugar constituents which make it a promising biocatalyst for large scale ethanol production [1]. Effective scale-up is essential for successful bioprocessing. The design of microbial processes depends on the product, microbial strain, growth conditions, bioconversion/biotransformation conditions and bioreactor geometry. Hence, for a given product, an adequate and comprehensive approach has to be established that includes the detailed characterization of the process parameters which directly linked to the product yield. A particular scale-up strategy is carried out by maintaining a specific set of parameters constant throughout the scale-up process, in order to ensure success of the production [2]. However, this is quite complex since there are several parameters influencing transport phenomena and dynamics within a bioreactor. Moreover, these parameters are directly related to mass transfer, mixing, power input, bulk rheology and shear induced by agitation or aeration, substrate and products concentration, nutrients and microconditions in the reactor [3]. Among several scale-up parameters, impeller tip speed is the most common parameter. As a rule of thumb it is known that microbial damage may occur at tip speeds above 3.2 m/s and this number depends on many factors such as broth rheology [4]. If the scale-up is carried out using constant tip speed, the volumetric power consumption is often lowered, which can adversely affect the bioconversion. In many processes, oxygen behaves as a limiting substrate thus constant oxygen mass transfer coefficient can be used as scale-up parameter. The mass transfer of oxygen into the bulk is influenced by several variables, such as physical properties of the fluid, operational conditions and geometry of the reactor. The oxygen transfer rate can be kept constant by altering stirring speed which concomitantly alters the power input. The use of constant oxygen mass transfer coefficient as scaling criterion is widely applied in conventional scales from laboratory to large scales if there is a strong link between cell growth and dissolved oxygen tension profiles [5]. However if the fermentation broth is viscous, constant mixing time is used through scaling. Mixing time is defined as the time required for the reactor composition to achieve a specified level of homogeneity following addition of a tracer pulse at a single point in the vessel [6]. Mixing time contains some information on flow and mixing within the reactor and can be useful for the scale-up of growth-regulated products.

In this study bioethanol production using quince pomace and scaling up was investigated. Cemeroğlu et al. [7] reported that the quince pomace was directly used as a substrate without any chemical pretreatment (such as acid or base hydrolysis) due to the availability of the sugars (mostly glucose and fructose) in the pomace for microorganisms. There are several studies on the utilization of the fruit pomaces such as apple pomace without pretreatment process for the bioconversion of value-added bioproducts [8-10]. In this study quince pomace as an agro-industrial biomass was used for bioethanol production under microaerated conditions, eliminating the pretreatment step. In our previous study, we reported that microaerated conditions enhanced bioethanol production via promoting sugar consumption [11]. Considering the increasing demand on ethanol utilization worldwide, a suitable scale-up technology with a suitable scale-up parameter for bioethanol production from E. coli KO11 needs to be identified. To this end, the two main objectives of the present study were: (i) to evaluate the use of constant impeller tip speed, mixing time and oxygen mass transfer coefficient as scale-up methodologies under laboratory conditions for the scale-up process from 2 L reactor to 8 L stirred-tank reactor, considering whether an increase in the bioethanol yield can be achieved, and (ii) to validate the kinetic parameters for better describing the behavior of E. coli KO11 during bioethanol fermentation from quince pomace.

Materials and Methods

Growth conditions

Recombinant E. coli KO11 (pLOI 1910) strain was provided by courtesy of Professor L.O. Ingram from University of Florida. Stock cultures were stored in 40% glycerol at −80°C. Seed cultures of KO11 were maintained on modified Luria-Bertani (LB) agar containing 5 g of NaCl, 5 g of yeast extract, 10 g of tryptone, 20 g of glucose, 15 g of agar, and 600 mg of chloramphenicol per liter and kept at 4°C.

For inoculation, 3 colonies were transferred into 250 mL flasks containing 50 mL LB medium supplemented with 60 g/L glucose. Seed cultures were incubated under static conditions for 16 hours at 30°C. Cells were harvested by centrifugation (5000 g, 5 min) and washed with the fermentation medium.

Substrate

Quince pomace was used as a substrate for ethanol production instead of glucose. Quinces were pressed and dried to constant weight at 70°C in pasteur oven (Mermert GmbH & Co. KG D-91126, Germany) to remove bound-water. Dried pomace was grinded to 0.1 mm in size.

The total carbon (C) and nitrogen (N) content of dried quince pomace were determined using the Barbano and Walkley-Black methods, respectively [12,13]. Total C and N content were detected as 34.5% and 0.23% of the total dry biomass, respectively. Quince pomace was also reported to compose of 28.8% glucose, 55.7% fructose and 10.1% sucrose based on dry mass [7].
The agitation rate was 370 rpm in 8 L reactor based on constant \( k \alpha \) and the oxygen transfer rate (OTR) which was determined as 5 mmol O\(_2\)/L/h, was calculated by Eq. 2 [17].

\[
OTR = \frac{dO_2}{dt} = k_2 \alpha (C^+ - C^i) - D_{O_2} \dot{X}
\]  

(2)

Mathematical equations of scale-up process for bioethanol production are presented in Table 1. Calculated process parameters are given in Table 2.

\begin{table}[h]
\centering
\caption{Equations of scale-up processes for bioethanol production}
\begin{tabular}{ll}
\hline
\multicolumn{2}{c}{Mathematical equations} \\
\hline
\( Re_i = \frac{D_i^2 N_i}{\eta} \) & (Eq. 8)  \\
\( N_p = \frac{P_g}{\rho N_i^2 D_i^3} \) & (Eq. 9)  \\
\( P_g = k \left( \frac{P_{21}^2 N_i D_i^3}{Q^{0.56}} \right)^{0.26} \) & (Eq. 10)  \\
\hline
\end{tabular}
\end{table}

Analytical measurements and calculations

Biomass was determined and validated by counting colony forming units, measuring absorbance and dry cell mass. Absorbance was measured at 600 nm (\( A_{600} \)) using Unicam-Helios-\( \alpha \) spectrophotometer, and the cell concentration was converted to g dry cell mass per liter (DCM/L) using the conversion factor of 0.33 g-DCM/L/\( A_{600} \), for \( E. coli \) KO11 [18].

Considering the cell death was negligible, maximum specific growth rate (\( \mu_{\text{max}} \)) was calculated (Eq. 3) [15].

\[
\mu = \frac{\ln X_2 - \ln X_1}{\Delta t}
\]  

(3)

where \( X_i \) is the final cell concentration, \( X_i \) is the initial cell concentration and \( \Delta t \) is the time required for the increase in concentration from \( X_i \) to \( X_f \).

Fermentation broth viscosity was measured twice at the beginning and at the end of the fermentation period by a rotational viscometer (Brookfield model DV-E, USA) with LVtype spring torque using LV1 (61) spindle and determined by Poiseuille equation (Eq. 4). Average viscosity of 1.36x10\(^{-6}\) m\(^2\)/s was used in the equations.

\[
\frac{dV_B}{dt} = \pi D_C \frac{dP}{d\eta} \quad 8 \quad L
\]  

(4)

The density of the fermentation broth was measured by 25 mL pycnometer (Isolab, Germany) at the beginning and at the end of the fermentation and an average density value was used in the study (1033 kg/m\(^3\)).

Total soluble reducing sugar content of quince pomace was determined using dinitrosalicylic acid (DNS) method where the absorbance was measured at 540 nm [19].

Ethanol concentrations were measured using a Gas Chromatograph (6890N Agilent Technologies Network GC System) equipped with a flame ionization detector and a DB-FFAP 30 m×0.32 mm×0.25 mm capillary column (J&W Scientific) [20].

Ethanol yield (\( Y_{P/S} \)) was defined as the amount of ethanol produced per the amount of sugar consumed during fermentation (Eq. 5). Total ethanol yield against theoretical yield and volumetric productivity were calculated by the Eq. 6 and 7, respectively.

\[
Y_{P/S} = \frac{dE}{dS}
\]  

(5)

\[
\text{Total Ethanol Yield} = \frac{dE}{dS} \times \frac{1}{0.52} \times 10^3
\]  

(6)
decreased after 48 hours so the suitable process duration was chosen as 48 hours for the maximization of the volumetric productivity. Moreover, the ethanol concentration (p<0.05) obtained in 8 L stirred tank reactor was 19% higher than in 2 L stirred tank reactor at 48 hours based on constant \( t_m \) of scale-up. Similar to the ethanol production, the reducing sugar concentration decreased from 60 g/L to 4.52 g/L in 48 hours for 8 L stirred tank reactor and sugar consumption was 0.22 fold higher compared to 2 L stirred tank reactor based on constant \( t_m \) of scale-up (Figure 1a and Figure 1d). It was reported that using scale-up based on constant \( t_m \), bioethanol concentration of 70 g/L and 65 g/L, was obtained from sucrose and sugar beet juice in 18 hours, respectively using \( \text{Saccharomyces cerevisiae} \) IR2 immobilized on loofa sponge in 8 L [21]. In another study, 23 g/L ethanol was obtained from \( L. \text{japonica} \) hydrolysates by \( E. \text{coli} \) KO11 in 1 L reactor [22].

\[
Q_P = \frac{dE}{dt}
\]  

\text{(7)}

\text{Statistical analyses}

Statistical analyses of the collected data were performed by one-way analysis of variance (ANOVA). A probability value of \( p<0.05 \) was considered to denote a statistically significant difference of two batches. Data are presented as mean values±SEM (standard error of the mean).

\text{Results and Discussion}

\text{Scaling up based on constant} \( t_m \)

In this study, three common scale-up parameters (mixing time; \( t_m \), impeller tip speed; \( \omega_{tip} \) and oxygen mass transfer coefficient; \( k_{La} \)) of 8 L stirred tank reactor were compared with 2 L control reactor to obtain maximum ethanol yield. As seen in Figure 1a, the constant \( t_m \) experiment yielded a maximum bioethanol production of 23.42 g/L in 48 hours. The increase in bioethanol concentration gradually decreased after 48 hours so the suitable process duration was chosen as 48 hours for the maximization of the volumetric productivity. Moreover, the ethanol concentration (p<0.05) obtained in 8 L stirred tank reactor was 19% higher than in 2 L stirred tank reactor at 48 hours based on constant \( t_m \) of scale-up. Similar to the ethanol production, the reducing sugar concentration decreased from 60 g/L to 4.52 g/L in 48 hours for 8 L stirred tank reactor and sugar consumption was 0.22 fold higher compared to 2 L stirred tank reactor based on constant \( t_m \) of scale up (Figure 1a and Figure 1d). It was reported that using scale-up based on constant \( t_m \), bioethanol concentration of 70 g/L and 65 g/L, was obtained from sucrose and sugar beet juice in 18 hours, respectively using \( \text{Saccharomyces cerevisiae} \) IR2 immobilized on loofa sponge in 8 L [21]. In another study, 23 g/L ethanol was obtained from \( L. \text{japonica} \) hydrolysates by \( E. \text{coli} \) KO11 in 1 L reactor [22].

As seen in Figure 2a, the levels of log (CFU/mL) and the dry cell mass were 2.1% and 9.6% higher in 8 L stirred
In this study, power number was taken as 5.20 at fully turbulent flow for Rushton turbine impeller design equipped with baffles [4,23]. The characteristic empirical constant \((k)\) for a standard Rushton turbine impeller was taken as 10 [24]. Power consumption per unit volume \((P_o/V_L)\) is considered as a measure of mixing intensity and mass transfer rate. In this study, the increases in impeller tip speed and Kolmogorov eddy size from 2 L control reactor to 8 L stirred tank were considered as negligible, because the \(P_o/V_L\) ratio in the constant \(t_m\) experiment was shown lower increase than the constant \(v_{tip}\) and constant \(k_{La-ex}\) experiments (Table 2). This finding was in accordance with the study of [25].

Productivity is a significant parameter to determine the tank reactor than 2 L control reactor, respectively based on constant \(t_m\) experiment (p<0.05). This was due to the large surface area of the reactor, which led to more exposure to oxygen. The biomass concentration reached a maximum of 9.88 g/L around 48 h in scaling up based on constant \(t_m\) experiment (Figure 2a). These findings were compatible with the depletion of reduced sugar at the same point (Figure 1a).

As a rule of thumb, when the Kolmogorov eddy size becomes equivalent to the cell diameter or gets smaller, the movement of the flow lines can shear cells. In the scale-up study based on constant \(t_m\), the size of Kolmogorov eddies was calculated to be 2.6 x 10^-5 m which was considerably larger than an average E. coli cell size (Table 2). This could be attributed to the lower cell stress potential based on constant \(t_m\) experiment.

In this study, power number was taken as 5.20 at fully turbulent flow for Rushton turbine impeller design equipped with baffles [4,23]. The characteristic empirical constant \((k)\) for a standard Rushton turbine impeller was taken as 10 [24]. Power consumption per unit volume \((P_o/V_L)\) is considered as a measure of mixing intensity and mass transfer rate. In this study, the increases in impeller tip speed and Kolmogorov eddy size from 2 L control reactor to 8 L stirred tank were considered as negligible, because the \(P_o/V_L\) ratio in the constant \(t_m\) experiment was shown lower increase than the constant \(v_{tip}\) and constant \(k_{La-ex}\) experiments (Table 2). This finding was in accordance with the study of [25].

Productivity is a significant parameter to determine the
cost-effectiveness value of an industrial production. As seen in Table 3, scale-up based on constant $t_m$ results in the highest volumetric productivity of 0.49 g/L/h and the total ethanol yield of 78.67% with the highest sugar consumption rate (Figure 1a). It was shown that when the sugar consumption rate was increased, higher volumetric productivity of 0.42 g/L/h was observed for glucose supported LB medium with the total yield of 70% [26]. The ethanol production was achieved by Saccharomyces cerevisiae in 13 L semi-pilot scale production and the maximum ethanol concentration was 46 g/L for 10 days with the lower volumetric productivity of 0.015 g/L/h [27].

Scaling up based on constant $v_{tip}$

The maximum ethanol concentration was found to be 18.13 g/L in the constant $v_{tip}$ experiment which was 29.39% lower than obtained in the constant $t_m$ experiment in the 8 L stirred tank reactors (Figure 1a and Figure 1b). The amount of ethanol produced was considerably lower than all experiments based on constant $v_{tip}$ of scale up and this result may be attributed to the usage of the lowest agitation rate, which influences the mass transfer adversely for viscous fermentation broths of bioethanol production. Since sugar content is directly associated to ethanol production, these findings were also supported by the lowest sugar consumption of only 53.3% at the end of the fermentation in the constant $v_{tip}$ experiment (Figure 1b). During the production of ethanol from sugar beet juice by Saccharomyces cerevisiae IR2, it was concluded that the same or lower mixing rate used in 2 L bioreactor was not sufficient for 8 L bioreactor and consequently, the cells and the substrate were not uniformly distributed resulting in decreased ethanol productivity [21]. This can also be explained by the decrease of turbulent flow which was demonstrated by the decreases in $P_o/V_l$ values (Table 2). It was also emphasized that when a scale up procedure resulted in a few increased Reynolds number, a very low $P_o/V_l$ value is obtained, which is not sufficient for efficient mixing affecting product rate negatively [28]. Moreover, mixing time was 1.31 fold higher in 8 L stirred tank reactor than 2 L control reactor with the result of lower ethanol yield based on constant $v_{tip}$ experiment (Table 2). In other words, the longest mixing time was obtained in the constant $v_{tip}$ experiment and affected bioethanol yield unfavorably. These effects may be due to the phenomena that higher mixing time might influence the mass transfer adversely and causing probable death zones inside the reactor. It was reported that, the mixing of the fermentation broth and mixing time are important for the efficient operation in large scale of heterogeneous suspensions [29]. Longer mixing times can cause locally high sugar accumulation leaded to low dissolved oxygen levels [30,31]. In this study, the existence of death zones in 8 L reactor resulted lower biomass and product yields upon scale-up based on constant $v_{tip}$ (Table 3).

The biomass concentration based on constant $v_{tip}$ experiment was 15.15%, 21.21% and 10.6% higher than based on constant $t_m$, constant $k_\alpha$, and 2 L control reactor experiments, respectively (Figure 2a, 2b, 2c and 2d). These decreased levels of biomass growths in 8 L stirred-tank bioreactor of constant $v_{tip}$ experiment are attributed to the poor gas–liquid dispersion observed at the lower impeller speed with the higher Kolmogorov eddy size. It is worth noting that, as stirrer rate decreased, Kolmogorov eddy size increased throughout scaling-up for ethanol production by E. coli KO11 [18].

In this study, the minimum $\mu_{max}$ value of 0.22 (1/h) was obtained using scale-up strategy based on constant $v_{tip}$. Furthermore, ethanol yield was considerably lower when constant $v_{tip}$ parameter was applied than all reactors, as a result of insufficient mixing when using quince pomace as a viscous substrate (Table 3). The reduced ethanol yield in the scale-up experiments based on constant $v_{tip}$ is also likely to be the result of operating at the reduced agitation rate, and the poor gas–liquid dispersion.

Scaling up based on constant $k_\alpha$

In the scale-up studies based on constant $k_\alpha$ experiment, the ethanol concentration was 23.41% lower, whereas the biomass concentration was 9.59% higher than the scale-up studies based on constant $t_m$ at 48 hours (Figure 1c and Figure 2c). It is important to underline that the maximum $\mu_{max}$ value of 0.26 (1/h) was obtained using scale-up strategies based on constant $k_\alpha$ (Table 3). It was stated that scale-up based on constant $k_\alpha$ is more appropriate for aerobic processes or biomass production as cells may tend to produce more biomass which leads to decrease in product yields [30,32]. Aerobic K99 antigen production was successfully scaled up from 5 to 200 L by keeping $k_\alpha$ constant using recombinant E. coli MC1061 with a product yield of 0.03 g/g [33]. The shear stress was only 1.23 fold higher in 8 L stirred tank reactor of constant $k_\alpha$ experiment, corresponding to a shear stress of 4.23 N/m² in 2 L control reactor (Table 2). It was shown that shear stress with the value of 12.5 N/m² had no significant decrease on cell lysis or cell viability by the wild type of E. coli [34]. Constant $t_m$ was reported to be more applicable under microaerobic conditions compare to constant oxygen uptake rate for the scale-up of 2,3-butanediol fermentation by Enterobacter aerogenes in which homogeneity was important [35].

Since the $k_\alpha$ is a function of $P_o/V_L$, the Rushton turbine has a much larger mass transfer coefficient because it dissipates significantly more power than the other impellers studied at constant impeller speed [36]. The highest $k_\alpha$ value (1.2x10⁻² 1/s) was obtained in the constant $k_\alpha$ experiment at which also gave the best gas handling capacities shown by the highest $P_o/V_L$ ratio (Table 2).

Conclusion

In this study, it was shown that the best approach is the application of constant $t_m$ through scaling-up for viscous fermentation broths of bioethanol productions due to the
significance of the homogeneity. This work suggests that there is considerable potential from an economic perspective for using quince pomace waste as a substrate for bioethanol production since it is considered as waste for juice processing and cannot be used for further applications. The results obtained in this study will provide valuable guidelines for engineering of bioethanol producers.

Acknowledgements

This study was a part of Cost Action of the European Union, FP0602 and the authors wish to thank The Scientific and Technical Research Council of Turkey (TUBITAK) with the grant number of 107M650) for the financial support.

Conflict of Interest

There are no conflicts of interest among the authors.

References

[1] Dien B, Hespell R, Ingram L, Bothast R. Conversion of corn milling fibrous co-products into ethanol by recombinant Escherichia coli strains K011 and SL40. World J of Microbiol and Biotechnol 1997; 13:619-625.
[2] Schmidt FR. Optimization and scale up of industrial fermentation processes. Appl Microbiol Biotechnol 2005; 68(4):425-35.
[3] Burke F. Scale up and scale down of fermentation processes. Practical Fermentation Technol 2008:231-269.
[4] Villadsen J, Nielsen J, Lidén A. Bioreaction Engineering Principles 2011; pp. 548, Springer, New York.
[5] Gill NK, Appleton M, Baganz F, Lye GJ. Quantification of power consumption and oxygen transfer characteristics of a stirred miniature bioreactor for predictive fermentation scale-up. Biotechnol Bioeng 2008; 100(6):1144-55.
[6] Marques MP, Cabral J, Fernandes P. Bioprocess scale-up: quest for the parameters to be used as criterion to move from microreactors to lab-scale. J of Chemical Technol and Biotechnol 2010; 85:1184-98.
[7] Cemeroğlu B, Karadeniz F, Ozkan M. Fruit-vegetable Process Technologies-II (Turkish) 2001. Food Technologies Publications, Turkey.
[8] Hang Y. Production of fuels and chemicals from apple pomace. Food Technol 1987; 41:115-7.
[9] Hang Y, Lee C, Woodams E. A solid state fermentation system for production of ethanol from apple pomace. J of Food Sci 1982; 47:1851-2.
[10] Ngadi M, Correa L. Kinetics of solid-state ethanol fermentation from apple pomace. J of Food Engineering 1992; 17:97-116.
[11] Deniz I, Imamoglu E, Vardar-Sukan F. Aeration-enhanced bioethanol production. Biochemical Engineering J. 2014.
[12] Walkley A, Black IA. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 1934; 37:29-38.
[13] Barbano D, Rasmussen R, Lynch J. Influence of milk somatic cell count and milk age on cheese yield. J of Dairy Sci 1991; 74:369-88.
[14] Vant Riet K, Trampler J. Basic Bioreactor Design: Mixing 1991; pp. 183-220, Taylor & Francis Inc, New York.
[15] Bailey JE, Ollis DF. Biochemical Engineering Fundamentals 1986; pp. 984, McGraw-Hill Chemical Engineering Series, New York.
[16] Shuler ML, Kargi F. Bioprocess Engineering 2002; pp. 308, Prentice Hall, New York.
[17] Swartz JR. Scale-up of genetically engineered products. The World Biotechnol Report 1984; 368-91.
[18] Imamoglu E, Sukan FV. Scale-up and kinetic modeling for bioethanol production. Bioreossur Technol 2013; 14:311-20.
[19] Miller GL. Use of dinitrosalicilic acid reagent for determination of reducing sugar. Anal Chem 1959; 31:426-8.
[20] Azbar N, Tutuk F, Keskint K. Effect of organic loading rate on the performance of an up-flow anaerobic sludge blanket reactor treating olive mill effluent. Biotechnology and Bioprocess Engineering 2009; 14:99-104.
[21] Ogbonna JC, Moshima H, Tanaka H. Scale up of fuel ethanol production from sugar beet juice using loofa sponge immobilized bioreactor. Bioreossur Technol 2001; 76(1):1-8.
[22] Kim NJ, Li H, Jung K, Chang HN, Lee PC. Ethanol production from marine algal hydrolysates using Escherichia coli K011. Bioreossur Technol 2011; 102(16):7466-9.
[23] Nienow A. Agitators for mycelial fermentations. Trends in Biotechnology 1990; 8:224-33.
[24] Doran PM. Bioprocess Engineering Principles, 1995; pp. 439, Elsevier Academic Press, New York.
[25] Kossen N, Oosterhuis N. Modelling and scaling-up of bioreactors. Biotechnol 1985; 2:571-605.
[26] Tao H, Gonzalez R, Martinez A, Ingram LO, et al. Engineering a homo-ethanol pathway in Escherichia coli: increased glycolytic flux and levels of expression of glycolytic genes during xylose fermentation. J Bacteriol 2001; 183(10):2979-88.
[27] de la Roza C, Laca A, Garcia LA, Diaz M. Ethanol and ethyl acetate production during the cider fermentation from laboratory to industrial scale. Process Biochemistry 2003; 38:1451-6.
[28] Garcia-Ochoa F, Gomez E. Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. Biotechnol Adv 2009; 27(2):153-76.
[29] Zhang H, Baeyens J, Tan T. Mixing phenomena in a large-scale fermenter of starch to bioethanol. Energy. 2012; 48:380-91.
[30] Junker BH. Scale-up methodologies for Escherichia coli and yeast fermentation processes. J Biosci Bioeng 2004; 97(6):347-64.
[31] Enfors SO, Jahic M, Rozkov A, Xu B, Hecker M, et al. Physiological responses to mixing in large scale bioreactors. J Biotechnol 2001; 85(2):175-85.
[32] Pessoa Jr A, Vitolo M, Hustedt H. Use of f,k as a criterion for scaling up the inulinase fermentation process. Appl Biochemistry and Biotechnol 1996; 699-709.
[33] Wong I, Hernandez A, Garcia M, Segura R, Rodriguez I. Fermentation scale up for recombinant K99 antigen production cloned in Escherichia coli MC1061. Process Biochemistry. 2002; 37:1195-9.
[34] Lange H, Taillardier P, Riba JP. Effect of high shear stress on microbial viability. J of Chemical Technol and Biotechnol 2001; 76:501-5.
[35] Byun TG, Zeng AP, Deckwer WD. Reactor comparison and scale-up for the microaerobic production of 2,3-butanediol by Enterobacter aerogenes at constant oxygen transfer rate. Bioprocess Engineering 1994; 11:167-75.
[36] Sardeing R, Aubin J, Xuereb C. Gas-liquid mass transfer: A comparison of down-and up-pumping axial flow impellers with radial impellers. Chemical Engineering Research and Design. 2004; 82:1589-96.