Research article

Scaling-up fermentation of *Escherichia coli* for production of recombinant P64k protein from *Neisseria meningitidis*

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**ABSTRACT**

*Background:* P64k is a *Neisseria meningitidis* high molecular weight protein present in meningococcal vaccine preparations. The *lpdA* gene, which encodes for this protein, was cloned in *Escherichia coli* and the P64k recombinant protein was expressed in *E. coli* K12 GC366 cells under the control of a tryptophan promoter. P64k was expressed as an intracellular soluble protein about 28% of the total cellular protein. Several scale-up criteria of fermentation processes were studied to obtain the recombinant P64k protein at the pilot production scale. Results: The best operational conditions at a larger scale production of P64k recombinant protein were studied and compared using the four following criteria: Constant Reynolds number (*Re* constant), Constant impeller tip speed (*n* in *di* constant), Constant power consumption per unit liquid volume (*P/V* constant) and Constant volumetric oxygen transfer coefficients (*KLa/k* constant). The highest production of the recombinant protein was achieved based on the constant *KLa/k* scale-up fermentation criterion, calculating the aeration rate (*Q*) and the impeller agitation speed (*n*) by iterative process, keeping constant the *KLa/k* value from bench scale. The P64k protein total production at the 50 l culture scale was 546 mg l⁻¹ in comparison with the 284 mg l⁻¹ obtained at 1.5 l bench scale.

Conclusions: The methodology described herein, for the *KLa/k* scale-up fermentation criterion, allowed us to obtain the P64k protein at 50 l scale. A fermentation process for the production of P64k protein from *N. meningitidis* was established, a protein to be used in future vaccine formulations in humans.


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1. Introduction

*Neisseria meningitidis*, a gram negative diplococcus and an obligate human pathogen, causes significant morbidity and mortality in children and young adults worldwide through epidemic or sporadic meningitis and/or septicemia [1].

Immunization is the best and most efficacious preventive approach against meningococcal disease [2]. This is the reason why, first polysaccharide vaccines and later on conjugate vaccines against serogroups A, C, Y and W-135 meningococci were developed. However, most work on development of group B meningococcal vaccines has focused upon use of noncapsular surface components (i.e. lipopoligosaccharides (LOS) and outer membrane proteins, either individually- or as in proteoliposome vaccine preparations).

Several outer membrane proteins (OMPs) have been examined as vaccine candidates. Besides major OMPs, other surface proteins are under investigation in several laboratories, including highly conserved OMPs that could potentially protect against meningococcal disease in humans [3].

Among proteins present in outer membrane vesicles vaccine preparations, a high-molecular mass protein (P64k) was identified. This protein is common to many meningococcal isolates, and the *lpdA* gene encoding for this protein has been isolated and cloned...
in *Escherichia coli* for the further expression of the recombinant protein [4].

An extensive biochemical and biophysical characterization of the P64k protein has been carried out [5]. The protein has been described as a dihydrolipoamide dehydrogenase, based in its high degree of homology with two enzymes of the pyruvate dehydrogenase complex of several species. The first 110 amino acids are similar to those in the lipoyl-binding domain present in the enzyme dihydrolipoamide acetyl-transferase (E2), while the rest of the protein has homology with the enzyme lipoamide dehydrogenase (E3) [6,7]. The gene and protein have been sequenced and its 3D structure determined [8].

From the immunological point of view, the protein was first considered as a vaccine candidate against *Neisseria meningitidis* infection. The antigenicity of this protein was studied and sera from vaccines and convalescent patients were able to recognize the recombinant protein. At the same time, antibodies elicited in several animal models by the recombinant P64k protein recognized the natural protein, present in the whole cell meningococci and in outer membrane vesicle vaccine preparations [9,10,11]. However, the functional activity of antibodies elicited in several animal models and in human by the recombinant protein alone was not enough in terms of conferring protection against meningococcal disease to consider the protein as a unique vaccine candidate. On the other hand, taking into account that the recombinant protein was highly immunogenic [12,13], its function as an immunological carrier for peptides [14,15] and antigens [16,17] was evaluated. Recently, the P64k protein is employed as a recipient of anti-cancer vaccine candidate [18,19].

In order to satisfy the required quantities of P64k protein for the accomplishment of preclinical and clinical studies first, and subsequently for the use in these conjugate vaccines, it was necessary to scale up the fermentation process of the recombinant *E. coli* from 1.5 l [20] up to 50 l scale. Based on several scale up fermentation criteria, a comparative analysis was performed and the results are presented herein.

### 2. Materials and methods

#### 2.1. E. coli strain and expression plasmid

*E. coli* K12 GC366 [21] strain was transformed with the plasmid pM-152 [10], which contains the entire *lpdA* gene encoding for the P64k protein under the control of the tryptophan (*trp*) promoter [22].

#### 2.2. Growth conditions

The recombinant *E. coli* strain was grown at 37°C in 500 ml shake flasks (New Brunswick Scientific Co., USA) containing 100 ml Luria Bertani medium (LB) [21], supplemented with 10 g glucose l⁻¹, 100 μg tryptophan ml⁻¹ (Merck, Germany) and 50 μg kanamycin ml⁻¹ (Sigma Co. Ltd., USA) for 8 h and 150 rpm.

For growth in fermenters the minimal growth medium (MM) [21] supplemented with 10 g casein hydrolysate l⁻¹, 10 g tryptophane l⁻¹, 10 g glucose l⁻¹, 0.015 g CaCl₂·2H₂O l⁻¹, 0.246 g MgSO₄·7H₂O l⁻¹ and 50 μg kanamycin ml⁻¹, was used.

The bench scale was carried out in 2.5 l fermenters (B.H Marubishi Co., Ltd., Japan) with 1.5 l working volume and 37°C, 700 rpm, aeration rate 1.5 l min⁻¹, as fermentation parameters. The pH was controlled at 7.0 with 40% (v/v) NaOH or H₃PO₄ [20].

The scale-up batch process was optimized in 70 l fermenters (B.H Marubishi Co., Ltd., Japan) with 50 l working volume, at 37°C and pH 7.0, as fermentation parameters. The aeration rate and agitation speed varied depending of the studied scale-up criteria. The fermenter geometries for both working scales are shown in Table 1.

#### 2.3. Analysis

Four scale-up fermentation criteria were employed: (1) Constant Reynolds’s number (Re constant), (2) constant impeller tip speed (n di constant), (3) constant power consumption per unit liquid volume (P/V constant) and (4) constant volumetric oxygen transfer coefficients (KLa/k constant).

The cell dry weight, as cell growth correlation parameter, was determined using the Sartorius MA 30 moisture balance (Sartorius, Germany). The total protein concentration was measured by densitometry, on 12.5% SDS-PAGE of the recombinant *E. coli* whole cells.

### 3. Results and discussion

#### 3.1. Bench scale growth, expression and production of P64k recombinant protein

The typical schematic fermentation process at 1.5 l scale, for the production of the P64k recombinant protein is shown in Fig. 1.

During the first 8 h of growth at 1.5 l bench scale, an increase in the optical density (cell dry weight) was observed. The maximum value of the specific growth rate was determined during the first 2 h of growth (μ = 1.2 h⁻¹), being stabilized during the rest of the fermentation process at μ = 0.34 h⁻¹, when an increase in the expression of the recombinant protein was detected. After 8 h of fermentation, the specific growth rate began to decline, reaching values of μ = 0.06 h⁻¹ at 10 h of growth, when the fermentation process finished.

The P64k expression level and the protein production increased through the fermentation process, reaching at 10 h growth the highest productivity level.

At this scale, 2.6 g cell dry wt l⁻¹, 22% of P64k recombinant protein expression levels, 284 mg l⁻¹ of volumetric production and 28.4 mg l⁻¹ h⁻¹ productivity, were obtained. The biomass yield on initial glucose as substrate (Yₓ/s) and the product yield on this substrate (Yₓ/p) values were 0.26 g biomass/g substrate and 0.028 g product/g substrate, respectively.

The P64k expression level is comparable with those values obtained for other recombinant proteins produced in *E. coli* with a similar expression system, such as p36 (20%) [24] and gag24 (25%) from HIV [25] and Opf from *N. meningitidis* (32%) [26]. Likewise, the productivity levels, biomass yield on substrate and the product yield on substrate of P64k protein are in the same order as for the above mentioned recombinant proteins.

#### 3.2. Scale-up fermentation process of P64k recombinant protein

The scale-up parameters to determine the best operational conditions at a larger scale production of P64k recombinant protein were studied and compared using the four following criteria:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>1.5 l scale</th>
<th>50 l scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fermentation volume [m³]</td>
<td>0.0025</td>
<td>0.070</td>
</tr>
<tr>
<td>Working fermentation volume [m³]</td>
<td>0.0015</td>
<td>0.050</td>
</tr>
<tr>
<td>Vessel diameter (D) [m]</td>
<td>0.129</td>
<td>0.375</td>
</tr>
<tr>
<td>Impeller diameter (d) [m]</td>
<td>0.067</td>
<td>0.150</td>
</tr>
<tr>
<td>Static height of both (H) [m]</td>
<td>0.115</td>
<td>0.453</td>
</tr>
<tr>
<td>Number of impellers (N)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Geometric factor (fc)</td>
<td>0.61</td>
<td>0.92</td>
</tr>
<tr>
<td>Power number (Np)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Density (ρ) [kg m⁻³]</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

### Table 1

Geometry of fermenters employed in the scale-up processes.
Constant Reynold's number (Re constant), (2) Constant impeller tip speed (n di constant), (3) Constant power consumption per unit liquid volume (P/V constant) and (4) Constant volumetric oxygen transfer coefficients (KLa/k constant).

3.2.1. Scale-up under constant Reynold's number criterion

This scale-up procedure was performed using the Reynold's equation, Re = \( \rho \cdot n \cdot d_i^2 \cdot \mu \) [27,28,29,30,31], where \( \rho \) is the fluid density, \( \mu \) is the viscosity, \( n \) represents the impeller agitation speed, and \( d_i \) the impeller diameter.

For this scale-up criterion, then:

\[
n_{50} = n_{1.5} \left( \frac{d_{i1.5}}{d_{i50}} \right)^2
\]

[Equation 1]

The aeration rate at the larger scale, considering a constant aeration number (Na = \( Q/n \cdot d_i^3 \)), was calculated as [27,29,32]:

\[
Q_{50} = Q_{1.5} \left( \frac{n_{1.5}}{n_{1.5}} \right) \left( \frac{d_{i50}}{d_{i1.5}} \right)^3
\]

[Equation 2]

3.2.2. Scale-up under constant impeller tip speed criterion

The scale-up procedure using the constant impeller tip speed criterion [27,28,29,30,31] was performed according [Equation 3]:

\[
n_{50} = n_{1.5} \left( \frac{d_{i1.5}}{d_{i50}} \right)
\]

[Equation 3]

where, \( n \) represents impeller agitation speeds and \( d_i \) impeller diameters.

The aeration rate at the larger scale was calculated as previously define in [Equation 2].

3.2.3. Scale-up under constant power consumption per unit liquid volume criterion

This scale-up procedure, using the constant power per unit of volume criterion [27,28,29,30,31], is performed according the following equations:

\[
\frac{P}{V}_{1.5} = \frac{P}{V}_{50}
\]

[Equation 4]

\[
P = Np \cdot \rho \cdot n^3 \cdot d_i^3 \cdot f_c \cdot N
\]

[Equation 5]

\[
P_g = f_p P
\]

[Equation 8]

where, \( P \) corresponds to the non-gassed power, \( Np \) the power number, \( \rho \) the fluid density, \( n \) the impeller agitation speed, \( d_i \) the impeller diameter, \( N \) the number of impellents and \( f_c \) is the geometric factor.

The geometric factor was calculated as:\n
\[
f_c = \left( \frac{D}{H} \right)^{1/2} \left\{ \frac{1}{3} \left( \frac{d_i}{d_{i50}} \right)^3 \right\}
\]

where, \( D \) is the vessel diameter and \( H \) is the static height of broth. Substituting [Equation 5] in [Equation 4],

\[
n_{50} \left( \frac{d_{i1.5}}{d_{i50}} \right)^3 \left( \frac{d_{i50}}{d_{i1.5}} \right) \left( \frac{n_{1.5}}{n_{1.5}} \right) \left( \frac{f_{c1.5}}{f_{c50}} \right)^{1/3} = \frac{n_{1.5}}{n_{1.5}} \left( \frac{d_{i1.5}}{d_{i50}} \right)^2
\]

[Equation 6]

The aeration rate at the larger scale was calculated as previously defined in [Equation 2].

3.2.4. Scale-up under constant volumetric oxygen transfer coefficients KLa/k criterion

The implementation of constant KLa/k scale-up criterion was performed using the most successful correlations with dimensional equations of the form: KLa = k (Pg/V)\( \alpha \) (Vs)\( \beta \) [33], where k, \( \alpha \) and \( \beta \) are constants, Pg is the gassed power, V is the liquid volume and Vs is the superficial gas velocity. Taking into account that V = \( \pi \cdot D^2 \cdot H/4 \), where D is the vessel diameter and H is the static height of broth, and Vs = \( (4Q/\pi \cdot D^2) \), where Q the is the aeration rate, then.

\[
(K_{La}/k) = \left( 4 \frac{P_g}{\pi \cdot D^2} \right)^{0.4} \left( \frac{4 \cdot Q}{\pi \cdot D^2} \right)^{0.2}
\]

[Equation 7]

The gassed power factor \( f_p \) (fp = Pg/P), where P corresponds to the non-gassed power, is determined from the power requirements for agitation in a gassed system [27,28,29,30,31].

The value for the gassed power factor \( f_p \) is taken from the experimental correlations between Pg and operating variables presented as curves of Pg/P vs aeration number (Na), where Na = \( (Q/n \cdot d_i^3) \), being Q the aeration rate, n the rotation speed of impeller and \( d_i \) the impeller diameter.

Therefore, from the gassed power factor,
and the non-gassed power calculated as:

\[ P = N_p \rho m^3 d_i N_f c \]  

[Equation 9]

where \( N_p \) is the power number, \( \rho \) is the fluid density, \( n \) is the rotation speed of impeller, \( N \) is the number of impellers, and \( f_c \) is the geometric factor, calculated as \( f_c = (D H)^{1/2}/(3d_i) \), and substituting \[ f_c \] and \[ N_f \] in [Equation 7].

\[ K_{a/k} = \left( \frac{4}{\pi D^2} \right)^{0.6} \left( N_p d_i^3 N_f c p f_i / H \right)^{0.4} \left( n^{1.2} \right)^{0.2} \]  

[Equation 10]

According to this scale-up criterion, \( (K_{a/k})_{2.51} = (K_{a/k})_{2.51} \). The \( (K_{a/k})_{2.51} \) value of 8.7 was calculated from experimental conditions having the aeration rate \( (Q) \) of 1.5 min\(^{-1}\) and the impeller agitation speed \( (n) \) of 700 rpm at 1.5 l fermentation scale. The \( n \) and \( Q \) values for the 50 l fermenter conditions were calculated by iterative process using the [Equation 10], keeping a constant factor of 8.7 for \( (K_{a/k})_{2.51} \). The calculated values for the scale-up processes are shown in Table 2.

Using the calculated fermentation parameter combinations of aeration rate \( (Q) \) and the impeller agitation speed \( (n) \), three fermentation processes were performed for each condition. The average of \( P64k \) recombinant protein production is shown in Table 3. According to these results, the best combination of aeration rate and the rotation speed of impeller parameters to keep a constant \( K_{a/k} \) value of 8.7 was \( Q \) of 50 l min\(^{-1}\) and 320 rpm. This set of values was used to perform the comparison among the four scaling up fermentation processes.

The results of total \( P64k \) production related to the influence of aeration rate and the impeller agitation speed are shown in Fig. 2. The Fig. 2a represents the raw data from the fermentation processes according to Table 3. The highest level of \( P64k \) production is obtained in a range of 315–340 rpm of impeller agitation speed and 35–55 l min\(^{-1}\) aeration rates, having the maximum \( P64k \) production of 546 mg l\(^{-1}\), with 320 rpm agitation and 50 l min\(^{-1}\) aeration rates. A surface response graph for the \( P64k \) production is presented in Fig. 2b. This graph is characterized by a non-linear regression model, with \( Q \) (aeration rate) and \( n \) (impeller agitation speed) as independent variables [34]. The \( P64k \) production is represented in [Equation 11]:

\[
\text{Production} = -76.352.6315 + (904.934 Q) + (359.0292 n) - (2.2763 Q^2) - (2.172 Q n) - (0.4187 n^2)
\]

[Equation 11]

A positive influence of the aeration rate and the impeller speed, as independent variable parameters, over the theoretical total protein production is observed. This means, that at certain value of aeration rate with an increase of the impeller agitation speed, an optimal protein production might be expected. In concordance, at certain

<table>
<thead>
<tr>
<th>Variant no.</th>
<th>( Q ) [l min(^{-1})/vvm]</th>
<th>( n ) [rpm/pps]</th>
<th>( P/V ) [W/m(^2)]</th>
<th>( V_s ) [m/s]</th>
<th>( K_{a/k} ) [W(^{0.4}) m(^{-1}) s(^{-0.2})]</th>
<th>Production [mg l(^{-1})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10/0.17/0.2</td>
<td>420/7.00</td>
<td>5574.87</td>
<td>0.0015</td>
<td>8.7</td>
<td>277 ± 15</td>
</tr>
<tr>
<td>2</td>
<td>15/0.25/0.3</td>
<td>390/6.50</td>
<td>4643.54</td>
<td>0.0023</td>
<td>8.7</td>
<td>310 ± 23</td>
</tr>
<tr>
<td>3</td>
<td>20/0.33/0.4</td>
<td>370/6.17</td>
<td>3811.45</td>
<td>0.003</td>
<td>8.7</td>
<td>305 ± 38</td>
</tr>
<tr>
<td>4</td>
<td>25/0.42/0.5</td>
<td>360/6.00</td>
<td>3510.68</td>
<td>0.0038</td>
<td>8.7</td>
<td>293 ± 26</td>
</tr>
<tr>
<td>5</td>
<td>30/0.50/0.6</td>
<td>350/5.83</td>
<td>3226.16</td>
<td>0.0045</td>
<td>8.7</td>
<td>301 ± 30</td>
</tr>
<tr>
<td>6</td>
<td>35/0.58/0.7</td>
<td>340/5.67</td>
<td>2957.45</td>
<td>0.0053</td>
<td>8.7</td>
<td>325 ± 29</td>
</tr>
<tr>
<td>7</td>
<td>40/0.67/0.8</td>
<td>330/5.50</td>
<td>2583.02</td>
<td>0.0068</td>
<td>8.7</td>
<td>398 ± 49</td>
</tr>
<tr>
<td>8</td>
<td>45/0.75/0.9</td>
<td>320/5.32</td>
<td>2351.84</td>
<td>0.0083</td>
<td>8.7</td>
<td>430 ± 48</td>
</tr>
<tr>
<td>9</td>
<td>50/0.83/1.1</td>
<td>315/5.25</td>
<td>2063.62</td>
<td>0.0076</td>
<td>8.7</td>
<td>546 ± 51</td>
</tr>
<tr>
<td>10</td>
<td>55/0.92/1.1</td>
<td>315/5.25</td>
<td>2063.62</td>
<td>0.0076</td>
<td>8.7</td>
<td>405 ± 42</td>
</tr>
</tbody>
</table>

3.3. Comparison of all scale-up criteria

The scale-up process is one of the key points, not only in fermentation but also in the industry as a whole. In the real practice, several scale-up criteria have been studied and applied, but all of them based in empirical results [35,36].

The constant power input per unit volume of liquid (\( P/V \)) and constant volumetric oxygen transfer coefficient \( (K_{a}) \) are among the most scale-up fermentation criteria used in the industry [37,38,39]. No scale-up strategy as such is generally established, so for each process a suitable approach needs to be determined [27,32,40]. In practice, different methods are used for adequate scale-up of a bioreactor [41,42].

The parameters for the studied scale-up criteria and the influence over the \( P64k \) production are shown in Table 4. Scaling up with the constant Reynold’s number criterion, very low values for impeller agitation speed (20% regarding 1.5 l scale), \( P/V \) (4% regarding 1.5 l scale) and \( K_{a/k} \) (20% regarding 1.5 l scale) were obtained at 50 l scale. This might provoke an inappropriate mixing, resulting in around 50% lower values for cell dry weight and \( P64k \) production obtained at 1.5 l scale. This is in agreement that Reynold’s scaling up criterion is not a good one to use for such a fermentation process, since the degree of agitation decreases very strongly with the increase of the production scale [42,43,44,45,46].

The impeller tip speed scale-up criterion has some advantages in the case of bioprocesses with shear sensitive microorganisms or the absolute value of the impeller tip speed [42,47,48], but it is also argued that it is not a good enough criterion for scaling [45,49]. In our case, the cell dry weight and the final \( P64k \) production increased in 50%, compared to values obtained for Reynold’s scale-up criterion. To do so, an increase in the impeller agitation speed and the aeration rate of around 50% had to be implemented.

Maintaining a constant power per unit volume is another popular scale-up method, and has been successfully applied as a primary scale-up parameter for many antibiotic fermentations [40,42].
When scaled up with constant P/V criterion, similar values of cell dry weight and P64k production were obtained, as compared to those for the constant impeller tip speed criterion. This was observed in spite of the 30% increase of the impeller agitation speed and the aeration rates used for the constant impeller tip speed scale up criterion.

For instance, scale-up based on constant P/V resulted in an increase of the shear rate (calculated as: $n \cdot d_i$) of 30%, as compared with the constant impeller tip speed criterion and the $K_{La}/k$ scale up criterion. This was the highest value among all the studied criteria. With this criterion, a P/V value of 5.2 kW m$^{-3}$ was obtained, slightly higher than the range of values reported as a general rule (1 to 3 kW m$^{-3}$) [27,43]. This increase in the shear stress may correlate with an increase of the possible cell damage and therefore with the decrease of the final production of the protein of interest [45,50].

The volumetric mass transfer coefficient is the most often applied physical scale-up variable. It includes relevant parameters that influence oxygen supply such as agitation and aeration [36,40,51,52]. Therefore, a strong emphasis has been put on maintaining a constant $K_{La}$ in scale-up of process to ensure the same oxygen supply rate and to satisfy the oxygen demand of the desired cultivation. The same $K_{La}$ values can be achieved in different sized vessels by adjusting the operating conditions, i.e. stirrer speed and aeration rates on the larger scale to match the optimum $K_{La}$ conditions determined in the small-scale studies [53]. Several correlations estimating $K_{La}$ have been developed over the years for a range of different vessel sizes [33,37,54,55] and successfully scaled-up of processes from bench scale to pilot scale based on this criterion or in conjunction with another scale-up parameter have been established [56,57,58].

In this study, when the $K_{La}/k$ scale up criterion was used, assuming a constant value of 8.7 (calculated for the 1.5 l scale), the aeration rate increased in 5–6 times (50 l min$^{-1}$) as compared with values for the impeller tip speed (7.5 l min$^{-1}$) and P/V (9.8 l min$^{-1}$) criteria. However, a similar impeller agitation speed value was obtained when compared these three scale-up criteria. Using the parameters calculated for the $K_{La}/k$ scale up criterion, no shear increase occurred and the highest cell dry weight and P64k production was achieved.

Using the estimated parameters for the $K_{La}/k$ scale up criterion ($n = 320$ rpm, $Q = 50$ l min$^{-1}$), a total of 10 fermentation processes were

![Fig. 2. Schematic representation of results from the scale up variants using $K_{La}/k$ criteria. (a) Influence of aeration rate and agitation speed on the final production of P64k protein. The production of the recombinant P64k protein (■) is expressed in [mg l$^{-1}$], the aeration (●) is expressed in [l min$^{-1}$] and the agitation speed (▲) is expressed in [rpm]. (b) Surface response graph from this analysis (n: agitation speed, Q: aeration rate).](image)

<table>
<thead>
<tr>
<th>Parameters for comparison of scale-up fermentation criteria.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Parameters</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>$n$ [rpm/rps]</td>
</tr>
<tr>
<td>$P$ [W]</td>
</tr>
<tr>
<td>$Re$</td>
</tr>
<tr>
<td>$n \cdot d_i$</td>
</tr>
<tr>
<td>$P/V$ [W m$^{-3}$]</td>
</tr>
<tr>
<td>$K_{La}/k$ [W$^{0.4}$ m$^{-1}$ s$^{-0.2}$]</td>
</tr>
<tr>
<td>Dry weight [g l$^{-1}$]</td>
</tr>
<tr>
<td>P64k production [mg l$^{-1}$]</td>
</tr>
</tbody>
</table>

a Constant Reynold’s number scale-up criterion.
b Constant impeller tip speed scale-up criterion.
c Constant power consumption per unit liquid volume scale-up criterion.
d Constant volumetric oxygen transfer coefficients scale-up criterion.
performed. At the 50 l scale, during the first 6 h of growth an increase in the optical density (and the corresponding total cell dry weight) was observed. The maximum value of the specific growth rate was obtained during the first 2 h of growth ($\mu = 1.4 \text{ h}^{-1}$), being stabilized during the rest of the fermentation process at $\mu = 0.33 \text{ h}^{-1}$. After 8 h of fermentation the specific growth rate began to decline, reaching values of $\mu = 0.03 \text{ h}^{-1}$ at 10 h of growth.

The P64k expression level and the protein production increased through the fermentation process, reaching at 10 h growth the highest productivity level. As an average, at 50 l scale (using the $K_a/k$ scale up criterion), 3.9 g cell dry wt l$^{-1}$, 28% level of P64k carrier protein expression, 546 mg l$^{-1}$ volumetric production and 54.6 mg l$^{-1}$ h$^{-1}$ productivity were obtained. The biomass yield on initial glucose as substrate and the product yield on this substrate values were 0.39 g biomass/g substrate and 0.054 g product/g substrate, respectively. A behavior of these fermentation processes is shown in Fig. 3.

The total P64k protein production increased 1.9 folds, when data from both scale were analyzed. This augment is influenced by the 6% slightly increase in P64k protein expression and the 1.5 fold increase in the final cell growth. These values are comparable with those obtained for other recombinant proteins produced in E. coli under the same expression system such as gag24, gp-41 and p36 from HIV [24,59,60], and the Opc from N. meningitidis [26], where the proteins in study become “toxic” for the cell.

In conclusion, the methodology employed, using the $K_a/k$ scale-up fermentation criterion and calculating the aeration rate (Q) and the impeller agitation speed (n) by iterative process, keeping constant the $K_a/k$ value from bench scale, allowed us to obtain the highest P64k protein production at 50 l scale, in comparison with the other studied scale-up fermentation criteria. As a result, we have established a fermentation process for the production of the P64k recombinant protein from N. meningitidis, a protein to be used in future vaccine formulations in humans.

References
