Modelling, optimization and control of continuous two-stage Cephalosporin C production

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Abstract. Cephalosporin is one of the most consumed antibiotics for its effectiveness against a wide variety of infections. Most cephalosporin products are the semi-derivatives of Cephalosporin C (CPC), a metabolite of the fungus \textit{Acremonium chrysogenum}. Since naturally the desired metabolite is not produced in a large amount by the fungus, an innovative operational strategy is required to increase its yield for the production of the antibiotic to be economically feasible. One way to increase the cephalosporin productivity is by increasing the concentration of thin hyphae cell in the bioreactor, but this will lead to a higher blower power requirement for providing adequate availability of oxygen in the fermentation broth. Lack of oxygen will retard the growth rate and reduce the productivity. Conversely, excessive aeration of the fermentation broth will lead to high shear stress that can kill the cells. The present work investigates through dynamic simulation the effectiveness of a continuous two-stage aerobic fermentation for the CPC production. The operating conditions are optimized to determine an optimal trade-off between the cephalosporin productivity and blower power. An increase of the dissolved oxygen in the first bioreactor from 10\% to 20\% can increase CPC productivity by 75.5\% from 24.42 mg/L.hr to 42.86 mg/L.hr.

1. Introduction
Antibiotics are an important type of drugs that can kill or retard the growth of bacteria and help prevent bacterial infections. The demand for antibiotics is growing as bacterial infections are one of the continuous health problems that plague society. Among the antibiotics available on market today, cephalosporin is one of the types that are the most sold and consumed [1, 2]. Note that the fifth generation of the cephalosporin class antibiotics has been found capable of fighting off resistance strains of bacteria, for example, the methicillin-resistant \textit{Staphylococcus aureus} (MRSA) [3, 4]. Historically, the third generation of the cephalosporin was often used to treat community-acquired pneumonia.

Generally, the production of cephalosporin starts from the fermentation using a suitable fungus, for example, the most common one is \textit{A. chrysogenum} which can produce CPC. The CPC will then be treated \textit{via} chemical or enzymatic hydrolysis which transforms the CPC into 7-aminoccephalosporanic acid (7-ACA), which can subsequently be further processed into an antibiotic drug [5]. The exact type of a cephalosporin-based antibiotic that will be synthesized depends on the side chains attached to the 7-ACA. Figure 1 shows the difference in the molecular structures of these compounds as the CPC is turned into 7-ACA, and then from 7-ACA to a few different cephalosporin-based antibiotics.
Figure 1. Molecular structure of CPC, 7-ACA and Cephalosporin antibiotics.

The cost of antibiotic production at a commercial scale critically depends on the fermentation process step which requires an antibiotic-producing organism, usually a bacterium or fungus. In the fermentation process, the antibiotic molecules are secreted by the organism involved as its secondary metabolites. The antibiotics will then be separated from the fermentation broth before its subsequent use in antibiotic drug production. For an antibiotic drug to be economically feasible, manufacturers must be able to obtain a high yield of antibiotic from the fermentation process and be able to isolate them from the broth effectively. While antibiotics are produced naturally by microorganisms as their secondary metabolites, the desired metabolites are not often available in the quantities sufficient for large-scale production of antibiotic drugs. Therefore, it often requires an intensive effort to harvest enough amount of these secondary metabolites for mass antibiotic production. Bear in mind that some of the metabolites are produced in the late growth phase of the microorganism involved. This delayed production of desired secondary metabolites is due to inherent regulatory mechanisms of the given microorganism, i.e., the metabolites are suppressed in the logarithmic phase and only generated in low quantities in the stationary phase [6]. One of the reasons for this phenomenon is that the microorganism only needs a small amount of these secondary metabolites for their survival innately, as such the microorganism does not need to produce the metabolites in large quantity [7].

Although that may be true, with the right conditions the production of secondary metabolites can increase substantially. For example, the study by [8] has found that cultivating *A. chrysogenum* with different carbon sources will result in different growth rates and CPC production rates. Furthermore, the availability of sufficient oxygen in the growth environment is equally important for *A. chrysogenum* to produce CPC. Lack of oxygenation will lead to the production of penicillin N instead of CPC [9]. Thus, the determination of optimum conditions for the fermentation process is of prime importance for improving the antibiotic yield. Currently, the pharmaceutical industry is mainly using batch or fed-batch fermentation mode for the production of antibiotics. Nevertheless, the industry is slowly going through a transition towards a continuous production process because the benefits of continuous cultivation over batch fermentation can be realized through suitable operational strategies [10]. The advantages of the continuous process of antibiotic production are higher productivity, lower manufacturing cost, lower waste generation, higher efficiency, lesser energy needs and reduced human errors.

But the transition mentioned above will not be easy as there are still several challenges and research gaps to be resolved [11, 12]. These include the challenge of maintaining the stability of the fermentation
operation and biological system over a long period and the challenge of keeping the whole system sterilized as continuous fermentation is prone to contamination even in a closed system. A continuous fermentation process will also require more complicated equipment and process design. In addition, there is a gap in terms of insufficient research on the process dynamics and control of the continuous antibiotics production system. Moreover, the diauxic behaviour that \textit{A. chrysogenum} possesses during fermentation with inverted sugar might pose a difficulty to control using the conventional control system.

Several studies have been reported in the open literature to increase CPC productivity. For example, Liu \textit{et al.} [13] showed that the CPC productivity in \textit{A. chrysogenum} can be substantially increased by using a recombinant strain of the fungus. In another study, Shahidzadeh \textit{et al.} [14] showed that the addition of magnetite nanoparticles into the fermentation broth can increase the CPC production by 60%. Also, it has been reported that the fermentation production of the CPC can be increased two-fold using algal sugars from the third-generation biomass and glycerol [15]. Zafira and Nandong [16] demonstrated that the feeding strategy using two sugars (glucose and sucrose) in a fed-batch fermentation can be optimized and resulted in the maximum CPC productivity of 65.36 mg/hr. In this study, the idea behind the optimal operating strategy is to maximize the production of the thin cells by adjusting the glucose and sucrose flow rates into the bioreactor.

This study aims to modify a fed-batch model of CPC production into a continuous model, and then creating a MATLAB/Simulink simulation from the continuous model, in which a practical control strategy will be applied. Afterwards, Response Surface Methodology (RSM) is performed to determine the relationship between the various parameters and various factors that might affect the CPC productivity and blower power for the system. Being adopted previously to improve the production of antibiotics successfully in several \textit{Streptomyces} species, RSM has proven to be a useful tool to optimize the parameters of microbial fermentation through mathematics and statistics [17]. Besides that, the Weighted Sum Scalarization optimization approach is also used to combine the two objectives for maximizing CPC production and minimizing blower power requirement. Note that, so far, there has been very little research conducted on the production of CPC by \textit{A. chrysogenum} in a continuous two-stage fermentation process. The idea behind this production strategy is to maximize thin cell concentration through decoupling the thin and thick cell productions in separate but connected bioreactors.

2. Bioreactor modelling

2.1. Bioreactor model

Please note that the fermentation kinetic model used in the present work is obtained from the work of [18]. This kinetic model was derived based on a mixture of glucose and substrate where the former is first consumed by the fungus to produce thin filamentous hyphal cells. After the depletion of the glucose, the thin cells undergo a morphological transformation to thick cells while consuming the sucrose and producing the CPC. In the work of [18], the effect of oxygen limitation is not studied as the kinetic model ignores the oxygen limitation. Therefore, in the present work, the aforementioned kinetic model is further modified by including the dissolved oxygen (DO) term in the model. The kinetic data related to this dissolved oxygen (DO) term is obtained from the work of [19].

Figure 1 shows the schematic diagram of the two-stage continuous bioreactors used to produce CPC by \textit{A. chrysogenum}. The major purpose of the first bioreactor is to produce hyphae cells and to maximize the cell concentration before sending it to the second bioreactor. To repress the formation of thick cells, only glucose is fed into the first bioreactor. The hyphae cells from the first bioreactor will undergo morphological differentiation to thick cells and concurrently the CPC is generated and released into the fermentation broth. To maintain the growth of the thick cells while not repressing the CPC production, only sucrose is fed into the second bioreactor.
The mass balance for the liquid volume in the bioreactor 1:

$$\frac{d[V_1(1-\gamma_1)]}{dt} = F_a - F_b$$  \hspace{1cm} (1)

where the fraction of bioreactor holdup volume occupied by the cells is given by:

$$\gamma_1 = \frac{C_T}{\rho_{cell}}$$  \hspace{1cm} (2)

The total cell concentration, $C_T = C_h + C_t$ and $\rho_{cell} = 390.0$ g/L.

**Figure 2.** Schematic of aerated two-stage continuous fermentation for CPC production.

Hyphae cells:

$$\frac{d[V_1(1-\gamma_1)C_{h1}]}{dt} = -F_bC_{h1} + R_{h1}V_1(1 - \gamma_1)$$  \hspace{1cm} (3)

Thick cells:

$$\frac{d[V_1(1-\gamma_1)C_{t1}]}{dt} = -F_bC_{t1} + R_{t1}V_1(1 - \gamma_1)$$  \hspace{1cm} (4)

Glucose:

$$\frac{d[V_1(1-\gamma_1)C_{g1}]}{dt} = F_aC_{g0} - F_bC_{g1} + R_{g1}V_1(1 - \gamma_1)$$  \hspace{1cm} (5)

Enzyme:

$$\frac{d[V_1(1-\gamma_1)C_{e1}]}{dt} = -F_bC_{e1} + R_{e1}V_1(1 - \gamma_1)$$  \hspace{1cm} (6)

Cephalosporin C:

$$\frac{d[V_1(1-\gamma_1)C_{p1}]}{dt} = -F_bC_{p1} + R_{p1}V_1$$  \hspace{1cm} (7)

Notations: $C_{h1}, C_{e1}$ and $C_{g1}$ denote the concentrations of hyphae cells, enzyme and glucose in the bioreactor 1; $V_1$ is the bioreactor liquid volume, $C_{g0}$ inlet glucose concentration; $R_{h1}, R_{g1}$ and $R_{e1}$ denote the rates of hyphae cell growth, glucose consumption and enzyme production in the bioreactor 1. The mass balances for the oxygen in the liquid and gas phases are written as follows:

$$\frac{d[V_1(1-\gamma_1)C_{o1}]}{dt} = -F_bC_{o1} + V_1(1 - \gamma_1)(R_{otr1} - R_{our1})$$  \hspace{1cm} (8)

$$\frac{dy_{o1}}{dt} = F_{air1}y_{o0}/N_{t1} - R_{otr1}V_1(1 - \gamma_1)/N_{t1}$$  \hspace{1cm} (9)
Where $R_{otr1}$, $R_{our1}$, $C_{o1}$, $F_{air1}$, $y_{o1}$, and $N_{T1}$ denote the rate of oxygen transfer from gas to liquid phase, rate of oxygen uptake by the cells, dissolved oxygen concentration, air flow rate, mass fraction of oxygen in the headspace and total number of moles of air in the headspace respectively. Note that for the second bioreactor, the above mass balances are repeated with the streams as shown in Figure 2.

2.2. Rate equations

The rate equations are obtained from [18] and briefly shown as follows. Note that the rates have been modified by considering the limitation imposed by the dissolved oxygen concentration.

Rate of glucose consumption ($R_G$):

\[
R_G = \left( \frac{\mu_g}{Y_g} \right) C_T \tag{10}
\]

\[
\mu_g = \mu_{g_{\text{max}}} \left( \frac{C_g}{k_{g_{1}} + C_g} \right) \left( \frac{C_o}{k_o + C_o} \right) \tag{11}
\]

Rate of sucrose consumption ($R_s$):

\[
R_s = \left( \frac{\mu_s}{Y_s} + m_s \right) C_T \tag{12}
\]

\[
\mu_s = \theta_d \mu_{s_{\text{max}}} \left( \frac{k_{s_{2}} + C_s}{k_s + C_s} \right) \left( \frac{C_o}{k_o + C_o} \right) \tag{13}
\]

\[
\theta_d = \begin{cases} 
0 & \text{if } C_g > \epsilon \\
1 & \text{if } C_g \leq \epsilon 
\end{cases} \tag{14}
\]

Rate of hyphae cell growth ($R_h$):

\[
R_h = \mu_h C_T \cdot \left( \mu_i + \delta_h \right) C_h \tag{15}
\]

\[
\mu_h = \begin{cases} 
\mu_{g_{\text{max}}} & \text{if } C_g > \epsilon \\
\mu_{s_{\text{max}}} & \text{if } C_g \leq \epsilon 
\end{cases} \tag{16}
\]

\[
\mu_i = \frac{\mu_{s_{\text{max}}}}{1 + I_2 C_g} \tag{17}
\]

\[
\delta_h = \frac{\delta_{h_{\text{max}}}}{1 + I_1 C_g} \tag{18}
\]

In the current simulation the threshold glucose concentration is taken to be $\epsilon = 0.01$.

Rate of thick cell growth ($R_t$):

\[
R_t = \mu_t C_h - \delta_t C_t \tag{19}
\]

Rate of enzyme production ($R_e$):

\[
R_e = \alpha \mu_t C_h - \beta C_e \tag{20}
\]

Rate of Cephalosporin C production ($R_p$):

\[
R_p = C_e C_t - \sigma C_p \tag{21}
\]

\[
R_{otr} = k_{i.a} \left( C_{o_{eq}} - C_o \right) \tag{22}
\]

\[
R_{our} = R_{\text{max}} \left( \frac{C_o}{k_o + C_o} \right) C_T \tag{23}
\]

\[
C_{o_{eq}} = y_{o} P / H_o \tag{24}
\]
where $C_{o,eq}$, $P$ and $H_o$ denote the oxygen solubility in water, total pressure in the headspace and Henry constant for oxygen respectively. Note that the notations used in the above rate equations are the same as the notations used in [18]. Table 1 shows the parameter values used in the current modelling and simulation.

Table 1. Parameters used in the modelling and simulation obtained from [16,17].

| Parameters          | Values          |
|---------------------|-----------------|
| $\alpha$            | 9.0             |
| $\beta$             | 2.18787         |
| $\sigma$            | 0.01076         |
| $H_o \left( \frac{Pa \cdot L}{mol} \right)$ | $7.468 \times 10^7$ |
| $k_L.a \left( \frac{L}{hr} \right)$         | 162.0           |
| $k_{oa} \left( \frac{mmol-O_2}{L} \right)$ | $1.132 \times 10^{-4}$ |
| $R_{max} \left( \frac{mmol-O_2}{g-cell/hr} \right)$ | 1.31             |
| $Y_g \left( \frac{g-cell}{g-glucose} \right)$ | 0.46188         |
| $Y_s \left( \frac{g-cell}{g-sucrose} \right)$ | 0.4             |
| $I_1$               | 20.0            |
| $I_2$               | 300             |
| $\delta h_{max}$    | 0.00668         |
| $\delta t_{max}$    | 0.00441         |
| $\mu_{t_{max}}$     | 0.04526         |
| $\mu_{y_{max}}$     | 0.042           |
| $\mu_{s_{max}}$     | 0.021           |
| $m_x$               | 0.02267         |
| $K_{s1}$            | 0.1             |
| $K_{s2}$            | 10.199          |

2.3. Blower power equation

The blower power requirement is given by [20]:

$$H_P = 0.01542 \left( \frac{Q \cdot P_i}{\eta} \right) \left( \frac{P_d}{P_i} \right)^{0.283}$$

(25)

where $H_P$ the brake horsepower (bhp), $Q$ the blower inlet volumetric flow rate ($ft^3/min$), $P_i$ the blower inlet pressure (psia), $P_d$ the blower discharge pressure (psia), $\eta$ the blower efficiency and $X$ the blower adiabatic factor.

The total discharge pressure $P_{tot}$ (psig) from the blower is calculated as follows

$$P_{tot} = 0.433h_w + \Delta P$$

(26)

where $h_w$ the depth of water at top of diffuser (ft).

The blower discharge pressure is calculated as follows

$$P_d = P_{tot} + P_i$$

(27)

It is assumed that the average pressure drop is $\Delta P = 5$ kPa = 7.252 psi, across the liquid column in the bioreactor. Table 2 shows the parameter values used for the bioreactor design.
Table 2. Parameters of the bioreactor.

| Parameters                  | Values  |
|-----------------------------|---------|
| Bioreactor volume $V_R$ (L) | 1500    |
| Bioreactor diameter $D$ (m) | 1.0839  |
| Bioreactor height $H$ (m)   | 1.6258  |
| Pressure drop across liquid height $\Delta P$ (psi) | 7.2519 |
| Blower inlet pressure $P_i$ (psi) | 14.6959 |

3. Bioreactor control strategy

The CPC fermentation in the two-stage continuous bioreactors exhibits unstable dynamics. For this reason, it is important to control both bioreactors involved before one can optimize the CPC production performance. A simulation study reveals that to achieve stable continuous operation, it is important to control the liquid levels and dissolved oxygen concentrations in both bioreactors. In this process, controlling the liquid levels is the most important factor for the process stability based on the simulation study in this work; a drop or an increase in the liquid level in either bioreactor will severely affect the substrate conversion and hence the CPC productivity. Meanwhile, adequate availability of dissolved oxygen in the fermentation broth is crucial to the fungus growth rate.

When the dissolved oxygen concentration is too low, the growth rate will be severely inhibited and resulting in rapid cell decay; these combined effects will dramatically reduce the CPC productivity. On the other hand, if the dissolved oxygen concentration is too high, then this will cause a dramatic increase in the blower power requirement and hence increasing the cost of operation. Thus, there must be an optimum value of the dissolved oxygen concentration which ensure a sufficiently high growth rate while keeping the blower power requirement at an economic level. In addition to controlling the liquid levels and dissolved oxygen concentrations, it is recommended to control the glucose concentration in the first bioreactor and sucrose concentration in the second bioreactor to enhance the CPC productivity and minimize the unconverted substrates, i.e., saving the cost on substrates. In total, there are 6 proportional-integral (PI) control loops employed in the process (3 control loops for each bioreactor). Table 3 shows the controlled variables (CV), manipulated variables (MV) and their nominal values.

Table 3. Controlled variables, manipulated variables and the nominal values.

| MV  | CV       | Nominal MV value | Nominal CV Setpoint |
|-----|----------|------------------|---------------------|
| $F_{air_1}$ | $C_{o_1}$ | 302.1            | $5 \times 10^{-4}$  |
| $F_{air_2}$ | $C_{o_2}$ | 215.8            | $5 \times 10^{-4}$  |
| $F_h$   | $V_1$    | 6.8079           | 1100                |
| $F_d$   | $V_2$    | 10.247           | 1100                |
| $F_c$   | $C_{g_1}$ | 6.8079           | 3.0                 |
|        | $C_{s_1}$ | 3.4393           | 3.0                 |

The mathematical model of the continuous two-stage *A. chrysogenum* fermentation process presented in Section 2 earlier is implemented into MATLAB/Simulink, which is a software that provides a flexible dynamic simulation environment for analyzing dynamic and discrete systems. It is used widely within the industry and academia to represent process behaviour and control systems. The Simulink model, as shown in figure 3 below, consists several input and output signals into and out from the bioreactor blocks. Several PI controllers are used where the brief summary of the controllers have been discussed above.
Figure 3. Simulink model for the simulation of the two-stage fermentation process.

4. Results and discussion

4.1. Impact of bioreactor glucose and dissolved oxygen concentrations

The concentration of oxygen in the first bioreactor ($C_{O1,op}$) was found to be the limiting factor for the production of CPC. A graph of CPC productivity against $C_{O1,op}$ is shown in figure 4 below. The point that is labelled as 10% oxygen saturation is equivalent to 0.422 x 10^{-4} mmol/L, whereas the point labelled as 20% oxygen saturation is 0.844 x 10^{-4} mmol/L and 30% oxygen saturation is 1.265 x 10^{-4} mmol/L. Fairly similar to the results obtained in the study of [21], after the system surpassed the critical oxygen saturation level, the improvement of productivity due to the increase of oxygen slowly plateaued. In this case, the critical oxygen level is around 30%. The critical oxygen saturation level is defined as the onset beyond which an increase of dissolved oxygen level will lead to reduced rate of productivity.
Figure 4. Productivity against $C_{O1-sp}$.

From the simulation results (figures are not shown) obtained in MATLAB/Simulink, at a low oxygen saturation level, i.e., 10%, the concentration in the first bioreactor of hyphae cells ($C_{h1}$) and thick cells ($C_{t1}$) is around 84.3 g/L and 4.13 g/L respectively while in the second bioreactor, the concentration in the first bioreactor of hyphae cells ($C_{h2}$) and thick cells ($C_{t2}$) is at 15.5 g/L and 43 g/L respectively. The CPC concentration in the first bioreactor ($C_{p1}$) is at 49.25 mg/L whereas in the second bioreactor ($C_{p2}$), it is at 5150 mg/L. At a moderate oxygen saturation level, i.e., 20%, the $C_{h1}$ increased to 86.67 g/L whereas the $C_{t1}$ decreased to around 3 g/L. A lesser amount of CPC is also produced in the first bioreactor with $C_{p1}$ at only 28 mg/L. With fewer thick cells being formed prematurely, concentration for both type of cells along with CPC is increased in the second bioreactor. Specifically, the concentration of hyphae cells, thick cells and CPC are at 22.8 g/L, 43.86 g/L and 5870 mg/L respectively. Furthermore, there is more CPC biosynthesis enzyme in both bioreactors.

Given a higher dissolved oxygen saturation level, i.e., 30%, the $C_{h1}$ increased to 87.48 g/L while $C_{t1}$ further decreased to 2.53 g/L. In the meantime, the $C_{p1}$ dropped to 20.93 mg/L. Similar to the condition in the aforementioned system with different oxygen situation, the hyphae cells in the second bioreactor are also increased while the thick cells are decreased. In this case, the values ($C_{h2}$ and $C_{t2}$) are 27 g/L and 43 g/L respectively. On the other hand, the value for $C_{p2}$ is 5855 mg/L. Nonetheless, the concentration of CPC biosynthesis enzyme here in both bioreactors ($C_{e1}$ and $C_{e2}$) are higher than that of the bioreactors at 20% of oxygen level. Although the CPC concentration ($C_{p1}$ and $C_{p2}$) is lower in both bioreactors, the productivity of the system is still higher when compared with the system with 20% of $C_{O1-sp}$, i.e., dissolved oxygen concentration setpoint in the first bioreactor.

Meanwhile, the concentration of oxygen in the second bioreactor ($C_{O2-sp}$) has a lower impact on the overall productivity of the system. This is because the thick cells in the second bioreactor are originated from the filamentous hyphal cells in the first bioreactor. When the $C_{O2-sp}$ is increased from 0.36 x 10^{-4} to 1.2 x 10^{-4} mmol/L with the oxygen level being set at 10%, 20% and 30% of oxygen saturation, the productivity will increase by an average of merely 1.43 mg/hr.L. Particularly, at 10% oxygen saturation, the volumetric productivity is raised from 24.42 to 26.11 mg/h.L (an increase of 1.69 mg/hr.L); at 20% oxygen saturation, the volumetric productivity is raised from 42.86 to 44.19 mg/h.L (increase of 1.33 mg/hr.L); and at 30% oxygen saturation, the productivity is raised from 52.32 to 53.59 mg/hr.L (an increase of 1.27 mg/hr.L). In addition, it was found that the concentration of oxygen also affects the optimal glucose concentration setpoint in the first bioreactor ($C_{G1-sp}$). As the concentration of
oxygen is increased, the optimal glucose concentration setpoint \((C_{g\text{-sp}})\) is lower (figure 5). For illustration, as shown in figures 5, 6 and 7, the optimal \(C_{g\text{-sp}}\) for the first bioreactor with 10% oxygen saturation is around 5.5 g/L, while it is around 3.5 g/L for 20% oxygen saturation and 2.5 g/L for 30% saturation. As the oxygen saturation level is higher, the effect that arises from the changes in \(C_{g\text{-sp}}\) also become more apparent.

![Figure 5](image.png)

**Figure 5.** Productivity against \(C_{g\text{-sp}}\) under 10% oxygen saturation.

![Figure 6](image.png)

**Figure 6.** Productivity against \(C_{g\text{-sp}}\) under 20% oxygen saturation.

![Figure 7](image.png)

**Figure 7.** Productivity against \(C_{g\text{-sp}}\) under 30% oxygen saturation.
On the other hand, the effect of changing the sucrose concentration setpoint \((C_{\text{s,sp}})\) is minimal, and it is dependent on a variable like the \(C_{O_{1,sp}}\). When \(C_{O_{1,sp}}\) is at 10% saturation, productivity will increase following the increment of \(C_{s,sp}\) from 3 g/L up to 9 g/L. Any additional increase of \(C_{s,sp}\) after 9 g/L will result in the reduction of productivity from its peak value of 24.47 mg/hr.L. Contrarily when \(C_{O_{1,sp}}\) is at 20% and 30% saturation, productivity will decrease when \(C_{s,sp}\) is increased. In this case, a lower \(C_{s,sp}\) will result in higher productivity. For a 20% saturation \(C_{O_{1,sp}}\), the peak productivity value of 43.07 mg/hr.L occurs at \(C_{s,sp}\) of 0.5 g/L. Below that value, i.e. 0.1 or 0.25 g/L, the productivity is dropped to 43.06 mg/hr.L. For \(C_{O_{1,sp}}\) of 30% saturation, the peak productivity value of 53.43 mg/hr.L is achieved with \(C_{s,sp}\) of 0.02 g/L and below. After that, in any case that the value of \(C_{s,sp}\) is higher, the productivity will decrease.

4.2. Response surface models

Two response surface model were developed in which one is for the system at 20% oxygen saturation and another is for the system at 30% oxygen saturation. A total of 9 variables \((F_{\text{air,1}}, F_{\text{air,2}}, C_{O_{2,sp}}, V_{1,sp}, V_{2,sp}, C_{g,\text{in}}, C_{g,sp}, C_{s,\text{in}} \text{ and } C_{s,sp})\) were first screened to eliminate the variables that have insignificant effects on the system. Since the effects of all the variables are the same for both the system at different oxygen saturation level, the same variables \((C_{O_{2,sp}}, V_{1,sp}, V_{2,sp}, C_{g,\text{in}} \text{ and } C_{s,\text{in}})\) were included to develop the model. The factorial experimental design generated by the Design-Expert 12 software includes all independent variables as the factor with three levels; where 0.9 is 90% of the baseline value, 1 is the baseline value, and 1.1 is 110% of the baseline value. The experimental range and coded level of variables are given in table 4.

| Table 4. Experimental independent variables and their coded levels. |
|----------------|----------------|----------------|
| Independent variable | Symbol | Levels of coded variables |
|----------------|----------------|----------------|
| \(C_{g,\text{in}} \text{ (g/L)}\) | \(A\) | -1 | 0 | +1 |
| \(V_{1,sp} \text{ (L)}\) | \(B\) | 990 | 1100 | 1210 |
| \(C_{s,\text{in}} \text{ (g/L)}\) | \(C\) | 153 | 170 | 187 |
| \(V_{2,sp} \text{ (L)}\) | \(D\) | 990 | 1100 | 1210 |
| \(C_{O_{2,sp}} \text{ (10}^3 \text{ x mmol/L)}\) | \(E\) | 3.24 | 3.60 | 3.96 |

After the simulation was run and the response model is generated by Design-Expert 12, regression analysis and analysis of variance (ANOVA) were conducted for the model of productivity and blower power. The \(R^2\) values for both ANOVA analyses are very close to 1, where the adjusted and predicted \(R^2\) value for both models are in good agreement with each other, with a difference of less than 0.0002 between each other. The final regression equation obtained from the software in terms of coded factors for both models is shown below.

At 20% oxygen saturation the CPC productivity \((P_r)\) and blower power \((P_b)\) are given by:

\[
P_r = \{42.8 + 7.104A + 1.986B + 0.457C - 1.975D + 0.158E + 0.356AB + 0.094AC - 0.362AD - 0.019AE + 0.024BC + 0.338BD + 0.016BE - 0.028CD + 0.010CE - 0.007DE + 0.107A^2 - 0.269B^2 - 0.017C^2 - 0.074D^2 + 0.003E^2\} (28)
\]
\[ P_b = \{360.9 + 30.89A + 22.88B + 0.664C + 7.892D + 7.972E + 0.060AC \\
+0.513AD + 0.598AE - 0.007BC + 0.356BD + 0.201BE \\
+0.107CD + 0.095CE + 0.623DE + 2.596AB - 0.610A^2 \\
-0.070B^2 - 0.065C^2 - 0.151D^2 - 0.179E^2 \} \] (29)

At 30% oxygen saturation:
\[ P_{r} = \{52.3 + 8.761A + 1.542B + 0.541C - 1.533D + 0.186E + 0.320AB \\
+0.131AC - 0.013AE - 0.319AD - 0.008BC + 0.510BD \\
-0.004BE + 0.085CE - 0.003CD - 0.008DE + 0.159A^2 \\
-0.279B^2 - 0.071C^2 - 0.184D^2 - 0.034E^2 \} \] (30)

\[ P_b = \{444.7 + 40.49A + 36.72B + 0.625C + 8.589D + 8.424E + 3.537AB \\
+0.094AC + 0.603AD + 0.640AE + 0.013BC + 0.328BD \\
+0.188BE + 0.089CD + 0.109CE + 0.660DE - 0.492A^2 \\
-0.094B^2 - 0.085C^2 - 0.141D^2 - 0.184E^2 \} \] (31)

The most influential factor, i.e., see the coefficients of A in equations (28) and (30), is the inlet concentration of glucose in the first bioreactor \((C_{g,\text{in}})\), as according to figure 8 and 9, which shows that the slope for \(C_{g,\text{in}}\) are the steepest. This is because the increment of sugar in the bioreactor will promote the growth and propagation of cells. Moreover, swollen hyphae cells that produce CPC are derived from the filamentous hyphal cells, which are grown in the first bioreactor from being fed with glucose. With this being said, the enzyme that can stimulate the production of CPC will also be produced in a larger amount since there are more cells. Thus, increasing the inlet concentration of glucose is more effective than increasing sucrose. While glucose is used to build up the cell mass of the fungi, the role of sucrose is to let them attain the physiological state of CPC production and increase the CPC productivity with the amount of cell mass that is present [22]. For this reason, the concentration of swollen hyphae cells in the bioreactor will increase with the increment of \(C_{s,\text{in}}\). Small quantities of filamentous hyphal cells are also grown because of this. However, because of the low activity of the enzyme that hydrolyses sucrose, the sugar is harder to be assimilated and there is a limit to its consumption rate [23]. Therefore, sucrose is added in a smaller amount than glucose where its influence on the CPC productivity is also lower than that of glucose. Nevertheless, one thing to be concerned about is the solubility of the substrates in water, as the quantity of sugar added to the reactor must not exceed its solubility limit. Note that at 30 °C, 1 L of water can dissolve about 1200 g of glucose or 2195 g of sucrose. Figures 8 and 9 show the response surface plots of the productivity and blower power against several key parameters: liquid volume setpoint of the first and second bioreactors \((V_{1,sp} \text{ and } V_{2,sp})\), glucose inlet concentration \((C_{g,\text{in}})\) and sucrose inlet concentration \((C_{s,\text{in}})\).
At the same time, a high amount of glucose in the reactor might lead to excessive cells growth. This will prevent the cells from taking in enough oxygen as there will be a mass transfer limitation problem due to the filling up of bioreactor by the cells. Since the fermentation of *A. chrysogenum* is highly aerobic, it is necessary to solve this problem by inducing airflow using the blower. For this reason, the requirement for blower power is increased as well when the inlet glucose concentration is increased. However, this situation is undesirable as the increase of blower power will mean the increase in impeller agitation that can cause shear stress to the fungi and causes a breakage of the hyphae cells [24].

Following, the requirement for blower power will be increased as the working volume of the bioreactors get larger, so that aeration will be provided sufficiently for the extended area of the bioreactor. In the meantime, increasing the size of the first bioreactor can benefit the system, as more hyphal cells grow are allowed to grow, with fewer swollen hyphae cells and dead cells. Needless to say, more CPC biosynthesis enzyme is produced as well. Specifically, with the increase of the working volume, the dilution rate is slightly lower, and the residence time is longer. Conversely, a slightly higher dilution rate is needed in the second bioreactor as the productivity is improved when the working volume is decreased for the second bioreactor.

**Figure 8.** Response surface plots showing the effects of the interaction terms on productivity (Top left to bottom right: Productivity against $V_{1,sp}$ and $V_{2,sp}$; $C_{g,in}$ and $V_{2,sp}$; $C_{g,in}$ and $V_{1,sp}$; $C_{g,in}$ and $C_{s,in}$).
In the MATLAB/Simulink simulation, when the working volume is decreased in the second bioreactor, more filamentous hyphal cells are grown instead of the swollen hyphae cells. Simultaneously, the CPC concentration is decreased. However, more CPC biosynthesis enzyme was produced, and the overall CPC productivity of the system is slightly increased; this is expected from equations (28) and (30) where the coefficients of D in the equations are much smaller than that for A. Not to mention that the concentration of dead cells was also decreased. Thus, although the swollen hyphal cells are known to produce a larger amount of CPC, it was deduced that continuing to grow more filamentous hyphal cells in the second bioreactor may be beneficial for the system. On a side note, when the working volume for both bioreactors is increased or decreased by the same factor at the same time, the productivity will remain unchanged. This is because the beneficial effects of increasing the volume for the first bioreactor are being neutralized by the increment of volume for the second bioreactor and vice versa. The productivity of the system will only increase when the working volume for the first bioreactor is larger than the one for the second bioreactor.

Subsequently, the effects of changing the oxygen concentration in the second bioreactor on the system are significantly lesser than that of raising the oxygen in the first bioreactor. The impact is larger for changing the variable in the first bioreactor in this case since its effect is carried over to the second

Figure 9. Three-dimensional graph that shows the effects of the interaction terms on requirement of blower power (Top left to bottom right: Blower power requirement against $C_{g-in}$ and $V_{1-sp}$; $C_{g-in}$ and $V_{2-sp}$; $C_{O2-sp}$ and $C_{O2-sp}$; $C_{g-in}$ and $C_{O2-sp}$).
bioreactor. Thenceforth, it is observed from the simulation that increasing the oxygen can indeed increase productivity, but only by a small amount. This shows that the stage for the production of CPC requires less oxygen than the stage of growing filamentous hyphal cells. Increasing the oxygen will decrease the concentration of dead cells in the bioreactor and increase the amount of CPC biosynthesis enzyme in the bioreactor whereas decreasing the oxygen will do the opposite. All in all, it can be inferred that the CPC biosynthesis enzyme plays a critical role in the productivity of the system, since the fermentation of A. chrysogenum is an aerobic process with all rate-limiting enzymes [25].

4.3. Weighted objective scalarization for the multi-objective problem

Given that the optimization for this system is multi-objective, the weighted objective scalarization method will be used in addition to RSM. This approach transforms the multi-objective function into a scalar fitness function by multiplying each objective function with a weighting factor and summing them up altogether [26]:

\[ f(x) = \sum_{i=1}^{k} w_i f_i^{\text{norm}}(x), \quad \sum_{i=1}^{k} w_i = 1, \quad w_i \geq 0, \quad i = 1, ..., k \]  \hfill (32)

where \( w_i \) denotes the weighting factor (weight) that represent the relative importance of each normalised objective function. The higher value that the fitness function has, the more desirable is the solution.

As mentioned by [26], the normalisation of objective functions is required to make them scaled and dimensionless. In this case, the normalisation is done by using the following equations when the objectives are to be maximized or minimized:

\[ f_i^{\text{norm}} = 1 - \frac{\max(f_i) - f_i}{\max(f_i) - \min(f_i)} \]  \hfill (33)

for objectives to be maximized.

\[ f_i^{\text{norm}} = \frac{\max(f_i) - f_i}{\max(f_i) - \min(f_i)} \]  \hfill (34)

for objectives to be minimized.

The bounds of the objective function \( f_i, \max(f_i) \) and \( \min(f_i) \), is taken as the maximum and minimum value of the responses obtained from the simulation runs. Using the equations above, the value of the scalar fitness function is calculated by applying different sets of the weighting factor, from 0:1 to 1:0, with a difference of 0.1 between each combination of weights, to the productivity and blower power objective function respectively. Productivity will be the objective function to be maximized while blower power will be the objective function to be minimized. When the weighting factor is set to 0:1, it means that the solution is focused on reducing the requirement for blower power with little care given to the yield of the product. Vice versa, when the weighting factor is set as 1:0, it means that the solution is focused on increasing the yield of the product while giving less attention to the requirement of blower power for the process.

The values of fitness functions that are calculated from using different sets of the weighting factor, are inserted as responses into the response surface model that was generated by the Design-Expert 12 software, to perform optimization. The optimized operating conditions in coded factors for a different set of weighting factors along with their predicted responses are tabulated in table 5 and 6 for the system with 20 % oxygen saturation and 30 % oxygen saturation respectively.
Table 5. Optimal operating conditions of the system with 20 % oxygen saturation.

| Weightage | $C_{g\text{in}}$ | $V_{1\text{sp}}$ | $C_{s\text{in}}$ | $V_{2\text{sp}}$ | $C_{O2\text{sp}}$ | Productivity (mg/L.hr) | Blower Power (hp) |
|-----------|------------------|------------------|------------------|------------------|-----------------|----------------------|------------------|
| 0:1       | 0.900            | 0.900            | 0.901            | 0.900            | 0.900           | 35.295               | 288.659          |
| 0.1:0.9   | 0.900            | 0.900            | 0.930            | 0.900            | 0.900           | 35.409               | 288.846          |
| 0.2:0.8   | 0.900            | 0.900            | 1.100            | 0.900            | 0.900           | 36.007               | 289.590          |
| 0.3:0.7   | 0.908            | 0.900            | 1.081            | 0.901            | 0.909           | 36.538               | 292.575          |
| 0.4:0.6   | 1.086            | 0.900            | 1.065            | 0.901            | 0.900           | 49.225               | 340.271          |
| 0.5:0.5   | 1.095            | 0.900            | 1.100            | 0.900            | 0.900           | 50.057               | 342.620          |
| 0.6:0.4   | 1.100            | 0.900            | 1.100            | 0.900            | 0.900           | 50.452               | 343.957          |
| 0.7:0.3   | 1.100            | 1.011            | 1.100            | 0.900            | 0.900           | 52.942               | 378.211          |
| 0.8:0.2   | 1.100            | 1.085            | 1.099            | 0.904            | 0.980           | 54.727               | 408.003          |
| 0.9:0.1   | 1.100            | 1.100            | 1.100            | 0.900            | 1.045           | 54.708               | 417.790          |
| 1:0       | 1.100            | 1.100            | 1.099            | 0.900            | 1.099           | 54.810               | 422.174          |

Table 6. Optimal operating conditions of the system with 30 % oxygen saturation.

| Weightage | $C_{g\text{in}}$ | $V_{1\text{sp}}$ | $C_{s\text{in}}$ | $V_{2\text{sp}}$ | $C_{O2\text{sp}}$ | Productivity (mg/L.hr) | Blower Power (hp) |
|-----------|------------------|------------------|------------------|------------------|-----------------|----------------------|------------------|
| 0.1       | 0.900            | 0.900            | 0.900            | 0.900            | 0.900           | 43.106               | 355.158          |
| 0.1:0.9   | 0.900            | 0.900            | 0.990            | 0.900            | 0.900           | 43.478               | 355.514          |
| 0.2:0.8   | 0.900            | 0.900            | 1.100            | 0.900            | 0.900           | 43.774               | 355.775          |
| 0.3:0.7   | 0.905            | 0.901            | 1.079            | 0.909            | 0.906           | 44.103               | 359.153          |
| 0.4:0.6   | 1.055            | 0.902            | 1.071            | 0.905            | 0.902           | 57.245               | 412.740          |
| 0.5:0.5   | 1.098            | 0.901            | 0.923            | 0.900            | 0.903           | 60.361               | 426.223          |
| 0.6:0.4   | 1.100            | 0.900            | 1.100            | 0.900            | 0.900           | 61.585               | 427.396          |
| 0.7:0.3   | 1.100            | 0.902            | 1.099            | 0.901            | 0.914           | 61.610               | 429.460          |
| 0.8:0.2   | 1.100            | 1.007            | 1.100            | 0.900            | 0.977           | 63.543               | 476.810          |
| 0.9:0.1   | 1.100            | 1.097            | 1.100            | 0.900            | 1.100           | 64.772               | 522.932          |
| 1:0       | 1.100            | 1.100            | 1.100            | 0.900            | 1.100           | 64.803               | 524.242          |
5. Conclusion
In this research, a mathematical model for the continuous two-stage fermentation of *A. chrysogenum* to produce CPC has been modified from a fed-batch fermentation reported in the literature, and a simulation of the two-stage process has been conducted in MATLAB/Simulink based on the modified mathematical model. For the control strategy, six PI controllers have been applied in the simulation. In addition, the operating conditions of the continuous fermentation process have been optimized using the Weighted Sum Scalarization and Response Surface Methodology (RSM). Using an equal weighting factor of the CPC productivity and blower power in the optimal operating conditions (refer to table 5 and 6), the optimal productivity increased by 17.1% when the dissolved oxygen concentration was increased from 20% to 30%. This led to an increase in the blower power by 24.4%. As the weighting factor placed is higher on the productivity and lower on the blower power (refer to table 5 and 6), the optimal productivity and blower power would increase. When weighting factor is all placed on the productivity (i.e., only the productivity was optimized), the productivity only increased by 7.4% from the value obtained when equal weighting factor were placed for the 30% dissolved oxygen concentration scenario in table 6. Meanwhile, the blower power increased by 23% which is a larger increase than that of the productivity. This result shows that it is important to consider both the productivity and blower power when optimizing the CPC production process. It was found in this study that as the dissolved oxygen concentration increased, the sensitivity of the fermentation performance towards mass transfer rate decreased. Without an oxygen saturation limit, the maximum productivity can be achieved with a low mass-transfer rate, but the blower power requirement would be impractically high. Also, it was found that changing the glucose concentration gave a higher impact than changing sucrose concentration on the CPC productivity and blower power requirement. It can be concluded that the application of continuous two-stage fermentation can increase the CPC productivity from that in batch fermentation.

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