Bioreactor design for the production of recombinant human therapeutic protein expressed in Pichia pastoris - A theoretical novel approach

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\textbf{ABSTRACT}

Bioreactors are the heart of any bioprocess operations. Currently there is a growing need for an efficient production of recombinant human therapeutic proteins worldwide. Most of the biotechnology based industries prefer \textit{Pichia pastoris} as a host system for producing recombinant human therapeutic proteins because of the high cell density and product yield obtained from this particular species. This paper describes the bioreactor design procedure for producing 10kg yr\textsuperscript{-1} of recombinant human follicle stimulating hormone through \textit{Pichia} pastoris. The protein used in our study is only a model for this work. Complete material and energy balance calculations were done in this study which is prerequisite for design calculations. It was calculated that for producing 10kg yr\textsuperscript{-1} of our model protein, a 26500L working volume bioreactor is required. Design calculations were carried out by fixing the \(k_La\) as 0.080 sec\textsuperscript{-1}. Mechanical design calculations were also performed in this work. The novel design calculation methods presented in this work is very easy to learn and easy for the designers to design an efficient bioreactor for producing any proteins or enzymes in an industrial scale by only changing the few variables such as the empirical formula of the microbe and product of interest, operating conditions etc.

\textbf{KEYWORDS}

Bioreactor; \textit{Pichia pastoris}; Therapeutic protein; Scale up; Material balance.

\textbf{INTRODUCTION}

Bioreactor refers to any device or system that supports a biologically active environment. Bioreactors are commonly cylindrical, ranging in size from liters to cubic meters, and are often made of stainless steel. On the basis of modes of operation, a bioreactor can be classified as batch, fed batch and continuous. Organisms growing in bioreactors may be suspended or immobilized. Bioreactor design is a relatively
complex engineering task, which is studied in the discipline of biochemical engineering. Under optimum conditions, the microorganisms or cells are able to perform their desired function with 100 percent rate of success. The bioreactor’s environmental conditions like gas flow rates, temperature, pH and dissolved oxygen levels, and agitation speed/circulation rate need to be closely monitored and controlled\[1\]. Most industrial bioreactor manufacturers use vessels, sensors and a control system networked together. There are 2 different methods followed to design a large scale bioreactor, either by theoretical calculations or by any of a number of commercial simulations. Though the commercial simulations are faster and accurate they are very costlier for license\[2\]. Hence, in this work the theoretical approach for bioreactor design was used with more care in calculation accuracy.

The production of recombinant therapeutic proteins began to be developed more than 25 years ago\[3,4\] with nowadays more than 300 protein products on the market or in late clinical stages\[5\]. The vast majority of these proteins have been produced in microbial (e.g., Escherichia coli, Saccharomyces cerevisiae) or mammalian systems (mainly Chinese Hamster Ovary cells). While prokaryotic systems are clearly superior in terms of ease of handling and high product yields, eukaryotes like yeast, mammalian cell lines are favoured when correct post-translational modification of the protein target is necessary\[5-8\]. Though mammalian cells are higher eukaryotes, they face a big problem of contamination and huge downstream processing steps in industrial scale. Pichia pastoris is one of the methylotrophic yeasts which are of both academic and industrial interest for the past one decade. It can grow in complex media and can be replicated in rich media. Pichia pastoris has a histidine-dependent methanol-inducible promoter. One of the drawbacks with Saccharomyces cerevisiae was that it did not have a strong inducible promoter. Pichia pastoris has a strong inducible promoter\[10\]. Considering the advantageous and wide industrial use of Pichia pastoris for the production of recombinant human therapeutic proteins, it has considered as a model organism in the present work for bioreactor design calculations. Moreover, we have considered human follicle stimulating hormone, a therapeutic protein which is used to treat human infertility as a model protein in our work. Our bioreactor design strategy can be employed for any protein or enzyme by replacing the empirical formula of the desired protein or enzyme in the calculation part. This case is applicable even for the microorganism selected in our work.

The method presented here is easy to learn, and offers a quick way to make preliminary estimates of the bioreactor design parameters.

**METHOD**

**Material balance**

The first step in a bioreactor design calculation is a material balance. Calculations were done initially for a shake flask fermentation conditions. The whole fermentation process was divided into 5 modes as shown in TABLE 1 & the material balance was done\[10\]. These 5 modes are already optimized for Pichia pastoris fermentation\[10\].

**Batch mode**

Considering glycerol as the carbon source and ammonium hydroxide as a nitrogen source for the initial growth of Pichia pastoris, and the empirical formula for Pichia pastoris (yeast) as $\text{CH}_{1.79} \text{O}_{0.5} \text{N}_{0.2}$, the following stoichiometric equation was developed\[11\].

$$\text{C}_3\text{H}_8\text{O}_3 + a\text{O}_2 + b\text{NH}_4\text{OH} \rightarrow c\text{CH}_{1.79} \text{O}_{0.5} \text{N}_{0.2} + d\text{CO}_2 + e\text{H}_2\text{O}$$

Here a, b, c, d and e are stoichiometric coefficients. The biomass yield for Pichia pastoris was taken as, $Y_{xs} = 0.4 \text{ g g}^{-1}$ and the percentage of ash content were taken as 8% in this calculation\[11,12\].

**Calculation of stoichiometric co-efficients**

$$c = 0.4 \times \frac{\text{Molecular weight of glycerol}}{\text{Molecular weight of biomass with ash}}$$

(1)
For calculating ‘a’, electron balance approach was used\[11\].

\[\gamma_s - 4a = c_\gamma_b\]  \hspace{1cm} \text{(2)}

where \(\gamma_s\) and \(\gamma_b\) are number of carbon atoms in substrate, degree of reduction for substrate and degree of reduction for biomass.

\(w = 3\)

\(\gamma_s = \frac{[3 \times 4 + 8 \times 1 + 3 \times (-2)]}{3} \Rightarrow 4.67\),

\(\gamma_b = \frac{[1 \times 4 + 1.79 \times 1 + 0.5 \times (-2) + 0.2 \times (-3)]}{1} \Rightarrow 4.19\)

Therefore, \(a = 0.6731\) mol CO\(_2\), \(b = 1.4327\) mol H\(_2\)O

For calculating ‘b’, Nitrogen balance approach was used\[11\].

\(b = 0.20c\)  \hspace{1cm} \text{(3)}

\(b = 0.2752\) mol NH\(_4\)OH/mol substrate

For calculating ‘d’, Carbon balance approach was used\[11\].

\(1.376 + d = 3\)

\(d = 1.624\) mol CO\(_2\)/mol substrate

Finally for calculating ‘e’, Hydrogen balance approach was used\[11\].

\(8 + 4b = 1.79 + 2e\)

\(e = 3.319\) mol H\(_2\)O/mol substrate

Hence the overall equation becomes,

\(C_3H_8O_3 + 2.06114O_2 + 0.2752NH_4OH \rightarrow 1.376CH_1.79O_0.5N_0.2 + 1.624CO_2 + 3.319H_2O\)

\(OD_{600}\) value for \(Pichia pastoris\) is 42 once it reaches the exponential phase\[10\].

By correlation\[13\],

\(\text{Wet Cell Weight (WCW)} = OD_{600} \times 0.22\)  \hspace{1cm} \text{(4)}

\(= 9.24\) gL\(^{-1}\)

Volume of basal salt medium taken= 1.5L

Volume of Trace Metal Solution (TMS) taken= 6.6mL

Volume of Inoculum taken= 150mL

Therefore, Total volume in the glycerol batch mode, \(V = 1.657\)L

Therefore, Total WCW for 1.657L = 9.24 g/L \times 1.657L = 15.31g

\(= 15.31/26.73 = 0.5704\)mol

1mol glycerol yields 1.376mol biomass therefore, moles of glycerol required to yield 0.5704mol biomass = 0.4145mol glycerol

2.06114mol O\(_2\) yields 1.376mol biomass therefore, moles of O\(_2\) required to yield 0.5704mol biomass= 0.8544mol O\(_2\)

0.2752mol NH\(_4\)OH yields 1.376mol biomass therefore, moles of NH\(_4\)OH required to yield 0.5704mol biomass = 0.1141mol NH\(_4\)OH

Therefore, the overall stoichiometric equation can be rewritten as,

\(0.4145C_3H_8O_3 + 0.8544O_2 + 0.1141NH_4OH \rightarrow 0.5704CH_1.79O_0.5N_0.2 + aCO_2 + bH_2O\)

By Carbon balance & Hydrogen balance, ‘a’ & ‘b’ values were calculated.

\(a = 0.6731\) mol CO\(_2\), \(b = 1.4327\) mol H\(_2\)O

Therefore, overall equation at the end the batch mode can be rewritten as,

\(0.4145C_3H_8O_3 + 0.8544O_2 + 0.1141NH_4OH \rightarrow 0.5704CH_1.79O_0.5N_0.2 + 0.6731CO_2 + 1.4327H_2O\)

**Fed-batch mode-I**

Fixed volume fed batch was considered for our calculations, where the limiting substrate is fed without diluting the culture. One of the advantage of this method is the culture volume can be maintained practically constant by feeding the growth limiting substrate in undiluted form and alternatively, the substrate can be added by dialysis without affecting the culture volume\[14\]. The following calculations were done to evaluate the parameters and stiochiometric coeffients in fed batch Mode I with glycerol as substrate.

Initial volume of fed batch, \(V_0\) (at the end of batch mode) =1.65L

Fed batch time, \(t_{fb}\) (from \text{TABLE 1}) =22.5 hr

Substrate volumetric flow rate, \(F\) (from \text{TABLE 1}) =24mL/hr\(^{-1}\)

Volume of trace metal solution added, \(V_{tms}\) =19.80mL

Fed batch volume, \(V_{fb} = F \times V_0 \times t_{fb} = 891\)mL

Total volume at the end of fed batch,

\(V_T = V_0 + V_{tms} + V_{fb}\)  \hspace{1cm} \text{(5)}

\(= 2561\)mL

Mass flow rate of the substrate, \(G = 12\)g L\(^{-1}\)hr\(^{-1}\)

\(\text{(Note)}: 100\)mL of 50\% glycerol has 50 g glycerol; therefore 24mL of 50\% glycerol has 12 g glycerol

Volume of 50\% glycerol added per liter of fermentation broth = 24mL L\(^{-1}\)hr\(^{-1}\)\times 22.5 hr = 540mL

Fed batch volume, \(V_{fb} = F \times V_0 \times t_{fb} = 891\)mL

Total volume at the end of fed batch,

\(V_T = V_0 + V_{tms} + V_{fb}\)

\(= 2561\)mL

\(\text{(Note)}: 50\% \text{ glycerol}= 50 \text{ g glycerol in 100mL of water}\)
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Therefore, Amount of 50% glycerol required,
\[ S = (891 \times 50)/100 = 445.50 \text{g} \]

Final amount of biomass formed at the end of fed batch-I,
\[ X_t = X_0 + X_{fb} \]
(6)

where, \( X_0 \) is the amount of biomass formed during the batch mode & \( X_{fb} \) is the amount of biomass formed only at the glycerol fed batch mode
\[
= (x_0 \times V_0) + (G \times Y_{xs} \times x_{fb}) \times V_t
\]
(7)

\[ = 291.84 \text{g} \]

The specific growth rate for fed batch mode I was calculated from the following equation\(^\text{[11]}\),
\[ \mu = \frac{G \times Y_{xs}}{x_0} \]
(8)

\[ \mu = 0.52 \text{ hr}^{-1} \]

Therefore, stoichiometric equation for this fed batch mode is given by,
\[ C_3H_8O_3 + aO_2 + bNH_4OH \rightarrow cCH_{1.79}O_{0.5}N_{0.2} + dCO_2 + eH_2O \]

The stoichiometric coefficients \( a, b, c, d, e \) and \( f \) were calculated by similar way as in Batch mode operation and the final stoichiometric equation for this mode was deduced to,
\[ C_3H_4O_{1.55} + aO_2 + bNH_4OH \rightarrow cCH_{1.79}O_{0.5}N_{0.2} + 0.863CO_2 + 3.1558H_2O \]
Mass balance was carried out and tabulated in TABLE 2.

Fed batch mode-II

In this fixed volume fed batch mode operation, methanol was used as a sole carbon source and ammonium hydroxide as a nitrogen source. Since, protein expression commences in \textit{Pichia pastoris} under methanol induction mode, the empirical formula for our model protein human follicle stimulating hormone was also considered in the product side of the stoichiometric equation. The empirical formula for the model protein was obtained from ProtParam\textsuperscript{TM} bioinformatics tool. The following calculations were made to evaluate various parameters and stoichiometric coefficients for fed batch mode II.

Initial volume of fed batch,
\[ V_0 (\text{at the end of fed batch mode I}) = 2.561 \text{L} \]
Fed batch time, \( t_{fb} \) (from TABLE 1) = 6 hr
Substrate volumetric flow rate,
\[ F \] (from TABLE 1) = 10.9mL L\(^{-1}\) hr\(^{-1}\)
Volume of trace metal solution added, \( V_{tms} = 30.732 \text{mL} \]

Fed batch volume,
\[ V_{fb} = F \times V_0 \times t_{fb} \]
(9)

Total volume at the end of fed batch,
\[ V_T = V_0 + V_{tms} + V_{fb} = 2.7592 \text{L} \]
Mass flow rate of the substrate, \( G = 10.9 \text{g L}^{-1} \text{hr}^{-1} \)
(Note: 100mL of 100% methanol has 100 g methanol; therefore 10.9mL of 100% methanol has 10.9 g methanol)

Volume of 100% methanol added per liter of fermentation broth= 65.4 mL L\(^{-1}\)
For 2.561L of fermentation broth, volume of 100% methanol added, = 65.4 mL L\(^{-1}\) \times 2.561 L = 167.49 mL
(Note: 100% methanol = 100 g methanol in 100 mL of water)
Therefore, Amount of 100% methanol required,
\[ S = 167.49 \text{g} \]

Final amount of biomass formed at the end of fed batch-II,
\[ X_t = X_0 + X_{fb} \]
(10)

where, \( X_0 \) is the initial amounts of biomass present at the start of fed batch-II & \( X_{fb} \) is the amount of biomass formed at the end of fed batch mode-II = 364.0207 g

Amount of Protein formed at this Fed Batch Mode-II,
\[ P = r_p \times t_{fb} \]
(11)

where \( r_p \) is the product formation rate which can be evaluated from the following relationship,
\[ r_p = \mu \times X_0 \times Y_{px} \]
(12)

where \( \mu \) is the specific growth rate which decreases with time and \( Y_{px} \) is the Protein yield w.r.t biomass. Specific growth rate can be calculated by the following relationship,
\[ \mu = \frac{G \times Y_{xs}}{x_0} \]
(13)

\[ = 0.0383 \text{ hr}^{-1} \]

10mg/L was assumed as the expression level of rh-FSH\(^14\) and used to calculate the protein yield, \( Y_{px} \) which was calculated as 7.1617 \times 10\(^{-5}\) g g\(^{-1}\) for this mode of operation. Therefore, stoichiometric equation for this fed batch mode including the empirical formula for our model protein in the product side is given by,
\[ C_3H_4O + aO_2 + bNH_4OH \rightarrow cCH_{1.79}O_{0.5}N_{0.2} + dCO_2 + eH_2O + fCH_{1.55}O_{0.27}N_{0.31} \]

The stoichiometric coefficients \( a, b, c, d, e \) and \( f \) were calculated and the final stoichiometric equation
for this mode was deduced to,
\[
\text{CH}_4 + 0.9983\text{O}_2 + 0.095793\text{NH}_4\text{OH} \rightarrow 0.4789\text{CH}_{1.79} \text{O}_{0.5} \text{N}_{0.2} + 0.52106\text{CO}_2 + 1.811\text{H}_2\text{O} + 4.12744 \times 10^{-5} \text{CH}_{1.55} \text{O}_{0.27} \text{N}_{0.31}
\]

Mass balance was carried out and tabulated in TABLE 3.

**Fed-batch mode-III**

The following calculations were made to evaluate various parameters and stoichiometric coefficients for fed batch mode III.

Initial volume of fed batch,
\( V_0 \) (at the end of fed batch mode II) = 2.7592 L

Fed batch time, \( t_{fb} \) (from TABLE 1) = 48 hr

Substrate volumetric flow rate, \( F \) (from TABLE 1) = 15 mL L\(^{-1}\) hr\(^{-1}\)

Volume of trace metal solution added, \( V_{tms} \) = 33.1104 mL

Fed batch volume,
\[
V_{fb} = F \times V_0 \times t_{fb}
\]
\[= 1986.624 \text{mL} \tag{14}\]

Total volume at the end of fed batch, \( V_T \) = 4.7789 L

Mass flow rate of the substrate, \( G \) = 15 g L\(^{-1}\) hr\(^{-1}\)

Volume of 100% methanol added per liter of fermentation broth = 720 mL

For 2.7592 L of fermentation broth, volume of 100% methanol added = 1986.624 mL

Therefore, Amount of 100% methanol required, \( S \) = 1986.624 g

Final amount of biomass formed at the end of fed batch-III,
\[X_t = X_0 + X_{fb} = 1740.341 \text{g} \]

Amount of Protein formed at this Fed Batch Mode-III, \( P = r_p \times t_{fb} \)
\[= 0.004548 \text{g hr}^{-1} \] \( \mu = 0.004548 \text{hr}^{-1} \)

\( \mu \) was calculated as 2.7564 \times 10^{-5} g g\(^{-1}\) for this mode of operation.

Therefore, \( r_p = (0.004548 \times 364.0207 \times 2.7564 \times 10^{-5}) \]
\[= 4.5634 \times 10^{-4} \text{g hr}^{-1} \]

Therefore, \( P_t = P_0 + (r_p \times t_{fb}) \)
\[= 0.02607 + (4.5634 \times 10^{-4} \times 48) \]
\[= 0.04797 \text{g} \]

Therefore, stoichiometric equation for this fed batch mode including the empirical formula for our model protein in the product side is given by,

\[
\text{CH}_4 + a\text{O}_2 + b\text{NH}_4\text{OH} \rightarrow c\text{CH}_{1.79} \text{O}_{0.5} \text{N}_{0.2} + d\text{CO}_2 + e\text{H}_2\text{O} + f\text{CH}_{1.55} \text{O}_{0.27} \text{N}_{0.31}
\]

The stoichiometric coefficients \( a, b, c, d, e \) and \( f \) were calculated and the final stoichiometric equation for this mode was deduced to,

\[
\text{CH}_4 + 0.9983\text{O}_2 + 0.095793\text{NH}_4\text{OH} \rightarrow 0.4789\text{CH}_{1.79} \text{O}_{0.5} \text{N}_{0.2} + 0.52106\text{CO}_2 + 1.811\text{H}_2\text{O} + 1.5892 \times 10^{-5}\text{CH}_{1.55} \text{O}_{0.27} \text{N}_{0.31}
\]

Mass balance was carried out and tabulated in TABLE 4.

**Fed-batch mode-IV**

The following calculations were made to evaluate various parameters and stoichiometric coefficients for this final fed batch mode IV.

Initial volume of fed batch, \( V_0 \) (at the end of fed batch mode III) = 4.7789 L

Fed batch time, \( t_{fb} \) (from TABLE 1) = 44 hr

Substrate volumetric flow rate, \( F \) (from TABLE 1) = 2 mL L\(^{-1}\) hr\(^{-1}\)

Volume of trace metal solution added, \( V_{tms} \) = 57.35 mL

Fed batch volume, \( V_{fb} = 420.5432 \text{mL} \)

Total volume at the end of fed batch, \( V_T = 5.257 \text{L} \)

Mass flow rate of the substrate, \( G = 2 \text{g L}^{-1} \text{hr}^{-1} \)

Volume of 100% methanol added per liter of fermentation broth = 88 mL

For 4.7789 L of fermentation broth, volume of 100% methanol added = 420.5432 mL

Therefore, Amount of 100% methanol required, \( S \) = 420.5432 g

Final amount of biomass formed at the end of fed batch-IV,
\[X_t = X_0 + X_{fb} = 1925.391 \text{g} \]

Amount of Protein formed at this Fed Batch-IV (Methanol), \( P = r_p \times t_{fb} \)
\[= 0.0022 \text{g hr}^{-1} \] \( \mu = 0.0022 \text{hr}^{-1} \)

\( \mu \) was calculated as 2.7303 \times 10^{-5} g g\(^{-1}\) for this mode of operation.

Therefore, \( r_p = (0.0022 \times 1740.341 \times 2.7303 \times 10^{-5}) \]
\[= 1.0454 \times 10^{-4} \text{g hr}^{-1} \]

Therefore, \( P_t = P_0 + (r_p \times t_{fb}) \)
\[= 0.04797 + (1.0454 \times 10^{-4} \times 44) \]
\[= 0.05257 \text{g} \]

Therefore, stoichiometric equation for this fed batch mode including the empirical formula for our model protein in the product side is given by,
tein in the product side is given by,
\[ \text{CH}_4 + a \text{O}_2 + b \text{NH}_4 \text{OH} \rightarrow c \text{CH}_{1.79} \text{O}_{0.5} \text{N}_{0.2} + d \text{CO}_2 + e \text{H}_2 \text{O} + f \text{CH}_{1.55} \text{O}_{0.27} \text{N}_{0.31} \]

The stoichiometric coefficients a, b, c, d, e and f were calculated and the final stoichiometric equation for this mode was deduced to,
\[ \text{CH}_4 + 0.99834 \text{O}_2 + 0.095785 \text{NH}_4 \text{OH} \rightarrow 0.4789 \text{CH}_{1.79} \text{O}_{0.5} \text{N}_{0.2} + 0.5211 \text{CO}_2 + 1.81083 \text{H}_2 \text{O} + 1.57352 \times 10^{-3} \text{CH}_{1.55} \text{O}_{0.27} \text{N}_{0.31} \]

Mass balance was carried out and tabulated in TABLE 5.
Therefore,
Total amount of biomass formed = 1925.391g
Total amount of protein produced in all the 3 methanol fed batch = 0.05257g

**Determination of bioreactor volume**

In-order to produce 10kg yr\(^{-1}\) of our model protein, the volume of bioreactor to be used has to be calculated. For calculating the bioreactor volume, the following method was adopted ASM manual of industrial microbiology and biotechnology\(^{[15]}\).

Targeted protein per year = 10kg yr\(^{-1}\)
Recovery factor\(^{[9]}\) = 75% (Since extracellular)
Therefore, required amount of annual target-protein to be produced = (10/0.75) = 13.333 kg yr\(^{-1}\)
Fraction of target protein present in the-secreted protein\(^{[9]}\) = 90%

Therefore,
Total protein to be produced = (13.333/0.9) = 14.814 kg yr\(^{-1}\)
To run one fermenter cycle, time taken= Fermentation time + Downtime= 141 hrs+10 hrs = 151 hr
Therefore no. of cycles to be run per year= (365\times 24)/151=58 cycles yr\(^{-1}\)
To produce 0.05257 g of protein per cycle-amount of biomass needed is = 1925.391 g
Therefore to produce 14814 g of protein per 58 cycles, (i.e., 255.414 g cycle\(^{-1}\))-amount of biomass needed is = 9354.61 kg biomass/cycle
\[
= 542567.38 \text{ kg biomass yr}^{-1}
\]
Final wet cell concentration = (1925.391 g / 5.257 L) = 366.253 g L\(^{-1}\)
\[
= 0.366253 \text{ kg L}^{-1}
\]

Total volume of broth = (542567.38 kg/year)/(Final wet cell conc.) =1481400.507 L yr\(^{-1}\)

**Assume one bioreactor**

Working volume = (1481400.507 L yr\(^{-1}\))/58 cycles = 25541.39 L

Actual volume = (25541.39 L)/0.7[30% head space is accounted] = 36487.7 L = 36500 L (approx)
Seed bioreactor volume = 36500\times 0.05
(Where 0.05 is volume ratio-Constant) = 1825 L
Pre-seed bioreactor volume = 1825\times 0.05 = 91.25 L
Inoculum development bioreactor volume = 91.25\times 0.05 = 4.563 L

**Bioreactor design**

The following datas were used as a standard data for the design calculations\(^{[10,16]}\).
Operating Temperature, T=30\(^{\circ}\)C
Viscosity, \(\mu = 25\times 10^{-3}\) kg m\(^{-1}\) sec\(^{-1}\)
Density, \(\rho = 1030\) kg m\(^{-3}\)
Bioreactor Volume, \(V = 36,500\ L\) (or) 36.5 m\(^3\)

\(Y_{02X} = 2.4145 \text{ g g}^{-1}\)

**Bioreactor design was divided into following 12 steps**

**Step 1**

\[
\text{OTR} = \text{OUR} \\
i.e., k_L a (C^* - C) = \mu \times Y_{02X} \times x_t
\]
Here \(\mu\) is the specific growth rate. This value is taken as 0.0022 hr\(^{-1}\) (which is lowest among other \(\mu\) values in the series of both batch and fed batch fermentations).
\(k_L a\)-Volumetric oxygen transfer co-efft in sec\(^{-1}\)
\(C^*:\)Equilibrium concentration of oxygen in mg L\(^{-1}\)
C-Bulk concentration of oxygen in mg L\(^{-1}\)
\(C^*\)was calculated from the below correlation,
\[
C^* = 14.161 - 0.3943 T + 0.007714 T^2 - 0.0000646 T^3
\]
\(C^* = 14.161 - 0.3943 (30) + 0.007714 (30)^2 - 0.0000646 (30)^3\)
\[C^* = 7.5304 \text{ mg L}^{-1}\]
The value of \(C\) is 10% of \(C^*\)\(^{[11]}\). Hence it was calculated as,
\[C = 0.75304 \text{ mg L}^{-1}\]
\(x_i\) is the final biomass concentration
\[x_i = X_i / V_f\]
\[x_i = 9354.61/25541.39\]
\[x_i = 366252.97 \text{ mg L}^{-1}\]
Therefore,
Therefore, \( v_{vm} = Q / V \)

Volumetric gas flow rate, \( Q \)
Gas velocity, \( v \)

Step 4

\[ k_{i} a = \left( \mu X_{O_{2}} X_{i} / (C' - C) \right) \]
\[ k_{i} a = (0.0022 \times 2.4145 \times 366252.97) / 6.7774 \]
\[ k_{i} a = 287.06 \text{ hr}^{-1} \]
\[ k_{i} a = 0.080 \text{ sec}^{-1} \]

Step 2

\[ V = (\pi / 4) \times (D_{T}^{3}) \times H_{L} \]
Where, \( V \) is the bioreactor volume.
\( D_{T} \) is the bioreactor diameter.
\( H_{L} \) is the height of liquid in the bioreactor.

Step 3

Agitator speed \( N \) was selected as 228rpm on trial and error basis

Step 5

Calculation of ungassed power:
\[ N_{Re} = 8 \times N \times (D_{i}^{3}) / \mu \]
\[ N_{Re} = 1030 \times 3.8 \times (1.3)^{2} / 25 \times 10^{-3} \]
\[ N_{Re} = 2.65 \times 10^{3} \]

From figure 1, \( N_{p} \), Vs \( N_{Re} \) by considering ruston turbine\[18\],
\[ N_{p} = 8 \]
But \( N_{p} = P / (\rho \times N_{o} \times (D_{i}^{3})^{3}) \)
Hence, \( P = N_{p} \times (\rho \times N_{o} \times (D_{i}^{3})^{3}) \)
\[ P = 8 \times 1030 \times (3.8)^{3} \times (1.3)^{3} \]
\[ P = 1678783.774 \text{ Watts} \]

Step 6

Correction for geometry
\[ F_{c} = \left[ (D_{T} / D_{i})^{3} \times (H_{L} / D_{i}) \right]^{1/2} / \left[ (D_{T} / D_{i}) \times (H_{L} / D_{i}) \right]^{1/2} \]
\[ F_{c} = 2.54 \times 3.308^{1/2} / 3 \times 3^{1/2} \]
\[ F_{c} = 0.97 \]

Therefore, corrected power,
\[ P_{c} = 0.97 \times P \]
\[ P_{c} = 0.97 \times 1678783.774 \]
\[ P_{c} = 1628420.261 \text{ W} \]

Step 7

Correction for number of impellers
\[ N_{f} = 2 \text{ (Impellers)} \]

Step 8

Correction for aeration

Flow number, \( N_{f} = (Q_{g} / V) \times (D_{i}^{3}) \)
\[ N_{f} = 0.2942 / (3.8 \times (1.3)^{3}) \]
\[ N_{f} = 3.524 \times 10^{-2} \]

From figure 2, \( P_{g} / P_{un} \) Vs \( N_{f} \) by considering flat blade turbine\[18\],
\[ (P_{g} / P_{un}) = 0.78 \]

Therefore, \( P_{g} = 0.78 \times P_{un} \)
\[ P_{g} = 2540335.607 \text{ W} \]

Step 9

Determination of \( k_{i} a \)
\[ k_{i} a = \left[ (0.0333 / (D_{i})^{3}) \times (P_{g} / V)^{0.541} \times (Q_{g}^{0.541} / \sqrt{D_{i}}) \right] \]
Here D is \( D_t \)
\[ k_m = 2.81 \times 10^{-4} \times 416.72 \times 0.6946 \]
\[ k_{oa} = 0.081 \text{ sec}^{-1} \]
Since the calculated \( k_{oa} \) and the target \( k_{oa} \) are more or less equal, the parameters which were assumed and calculated were accepted and the design is perfect. Moreover, 10\% deviation is accepted usually between calculated & target \( k_{oa} \).

**Step 10**

**Gas holdup & head space calculation**

\[ \Phi = \frac{(V_T - V_L)}{V_T} = 1.8 \times (P_m)^{0.14} \times (v_e)^{0.75} \]
\[ P_m = \frac{P_o}{(p \times V)} \]
\[ P_m = 2540335.607 / (1030 \times 36.5) \]
\[ P_m = 67.57 \text{ W kg}^{-1} \]
Therefore, \( [1-(V_L/V_T)] = 1.8 \times (67.57)^{0.14} \times (0.0344)^{0.75} \)
\[ V_T = 49.31 \text{ m}^3 \]
But, \( V_L = (\pi/4) \times (D_t)^2 \times H_L \)
\[ H_L = 49.31 \times (4/\pi) / (3.3)^2 \]
\[ H_L = 6.35 \text{ m} \]

During 10\% head space, \( H_L = 6.35 \text{ m} \)

**Step 11**

**Calculation of heat duty: (Energy balance)**

Heat released, \( Q = -Q_{\text{met}} - Q_{\text{shaft}} \)
\[ Q_{\text{shaft}} = \frac{P}{g} \]
\[ Q_{\text{shaft}} = 2540335.607 \text{ J/sec} \]
\[ Q_{\text{shaft}} = 2540335.607 \times 3600 / 4.184 \]
\[ Q_{\text{shaft}} = 218575.214 \text{ kcal hr}^{-1} \]
\[ Q_{\text{met}} = (\mu \times Y_{\text{O2X}} \times \chi) \]
Here maximum \( \mu \) value (fed batch glycerol phase) was considered.
\[ \mu = 0.52 \text{ hr}^{-1} \]
Therefore, \( Q_{\text{met}} = (0.52 \times 2.4145 \times 366.253) \)
\[ Q_{\text{met}} = 45.85 \text{ g L}^{-1} \text{ hr}^{-1} \]
\[ Q_{\text{met}} = 524516.41 \text{ gml hr}^{-1} \]
1 g mol of \( O_2 \) produces \( 460 \times 10^3 \text{ J energy} \),
Therefore \( 524516.41 \text{ gml hr}^{-1} \) of \( O_2 \) would produce,
\[ = 2.413 \times 10^6 \text{ KJ hr}^{-1} \]
\[ = 57672.08 \text{ kcal hr}^{-1} \]
Therefore, \( Q = -218575.214 - 57672.08 \)
\[ Q = 2.243 \times 10^6 \text{ kcal hr}^{-1} \text{ (or) } 2606.864 \times 10^3 \text{ J s}^{-1} \]

**To find amount of cooling water required**

\[ Q = m \times C^p \times \Delta T \]
Therefore, \( m = Q / (C^p \times \Delta T) \)

\[ C^p \text{ of water } = 75.4 \text{ J mol}^{-1} \text{ C}^{-1} \]
\[ \Delta T = (T_2 - T_1), \text{ where } T_2 \text{ is the water outlet temperature and } T_1 \text{ is the water inlet temperature.} \]
\[ \Delta T = (25-10) = 15\text{oC} \]
\[ \text{Hence, } = 2606.864 \times 10^3 / (75.4 \times 15) \]
\[ m = 2305 \text{ mol water sec}^{-1} \text{ (or) } 41.5 \text{ kg water sec}^{-1}. \]

**Step 12**

**Mechanical design: (for bioreactor)**

1) Cylindrical portion thickness, \( t_c = (P_i \times D_{o}) / [(2 \times f) - (P_i)] \)
where, \( P_i = 1.5 \times 10^5 \text{ Pa (Design pressure)} \)
‘f’ for S.S @ 30\text{oC} = 310 \times 10^6 \text{ Pa}
Joint Efficiency, \( J = 0.85 \)
Therefore, \( t_c = 0.94 \text{ mm} \)
2) Ellipsoidal head thickness, \( t_e = (P_i \times D_{o}) / [(2 \times fJ) - (P_i)] \)
Therefore, \( t_e = 0.94 \text{ mm} \)
3) Crown radius, \( R_c = 3.3 \times 10^3 \text{ mm} \)
4) Knuckle radius, \( R_k = 0.06 \times R_c = 198 \text{ mm} \)
5) Support thickness (Skirt) = usually 20-40 mm
6) Nozzle design: Fermenter working volume, \( V = 25.54139 \text{ m}^3 \)
Flow rate = \( V / t \)
‘t’ is the time required to fill \( = 45 \text{ min} \)
Therefore, flow rate = \( 25.54139 / 45 \times 60 \)
\[ = 9.46 \times 10^3 \text{ m}^3 \text{ sec}^{-1} \]
Nozzle area = flow rate / liquid velocity
Liquid velocity = \( 2 \text{ m s}^{-1} \)
Therefore, Nozzle area = \( 9.46 \times 10^3 / 2 \)
\[ = 4.73 \times 10^3 \text{ m}^2 \]
\[ (\pi/4) \times (D_n)^2 = 4.73 \times 10^3 \]
Hence \( D_n \) (Nozzle Diameter) = \( 77.60 \text{ mm} \)
7) Cooling coil requirement: From heat duty calculation, \( Q = 2606.864 \times 10^3 \text{ J/sec} \)
But \( Q = U \times A_1 \times (\Delta T_1) \), Where, \( U \) is the overall heat transfer coefft = \( 850 \text{ Wm}^{-2} \text{ K}^{-1} \) & \( \Delta T_1 \) is the logarithmic mean temperature difference
\[ = (\Delta T_1 - \Delta T_2) / \ln (\Delta T_1 / \Delta T_2) \]
\[ \Delta T_1 = (30-10) = 20 \text{oC} \]
\[ \Delta T_2 = (30-25) = 5 \text{oC} \]
Hence \( \Delta T_1 = 10.82 \)
Therefore, \( A_1 = 283.44 \text{ m}^2 \)
Available area \( A_2 = \pi \times D_{o} \times (H_L) \)
\[ A_2 = 60 \text{ m}^2 \]
\[ (\pi \times N_{o} \times D_{o} \times H_L) = (A_1 - A_2) \]
here ‘N_T’ is the number of cooling coil turns. 
\[ N_T = \frac{(283.44-60)}{\pi \times 3.3 \times 4.3} = 5 \text{ turns} \]

The summary of overall calculated results were tabulated in TABLE 6 and based on the results, a bioreactor was draw with 1:50 scale (Figure 3).

**RESULTS**

Material balance results for all the modes of opera-

![Figure 1: Standard graph for N_T Vs N_Re](image1)

![Figure 2: Standard graph for (P_g/P) Vs N_Q](image2)

A-Flat blade turbines, B-Vanned disc (8 vanes), C-Vanned disc (6 vanes), D-Vanned disc (16 vanes), E-Vanned disc (4 vanes), F-Paddle

**TABLE 1:** Standard fermentation conditions followed for *Pichia pastoris*

<table>
<thead>
<tr>
<th>Time (Hrs)</th>
<th>Stage</th>
<th>Mode</th>
<th>Feed substance</th>
<th>Feed rate (mL/L/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>Growth</td>
<td>Batch</td>
<td>None</td>
<td>Not applicable</td>
</tr>
<tr>
<td>20-42.5</td>
<td>Growth</td>
<td>Fed-batch</td>
<td>50% Glycerol</td>
<td>24</td>
</tr>
<tr>
<td>42.5-43</td>
<td>Starvation</td>
<td>batch</td>
<td>None</td>
<td>Not applicable</td>
</tr>
<tr>
<td>43-49</td>
<td>Induction</td>
<td>Fed-batch</td>
<td>100% Methanol</td>
<td>10.9</td>
</tr>
<tr>
<td>49-97</td>
<td>Production</td>
<td>Fed-batch</td>
<td>100% Methanol</td>
<td>15</td>
</tr>
<tr>
<td>97-141</td>
<td>Production</td>
<td>Fed-batch</td>
<td>100% Methanol</td>
<td>2</td>
</tr>
</tbody>
</table>

**TABLE 2:** Mass balance for Fed-Batch Mode-I

<table>
<thead>
<tr>
<th>Mass in</th>
<th>Mass out</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mol Glycerol = 92.0000g</td>
<td>1.3767 mol Biomass = 33.853g</td>
</tr>
<tr>
<td>2.0604 mol O_2 = 65.9328g</td>
<td>1.6233 mol CO_2 = 71.4252g</td>
</tr>
<tr>
<td>0.27534 mol NH_4OH = 9.6369g</td>
<td>3.456 mol H_2O = 62.208g</td>
</tr>
<tr>
<td>Total mass in = 167.57g</td>
<td>Total mass out = 167.49g</td>
</tr>
</tbody>
</table>

**TABLE 3:** Mass balance for Fed-Batch Mode-II

<table>
<thead>
<tr>
<th>Mass in</th>
<th>Mass out</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mol Methanol = 32.0000g</td>
<td>0.4789 mol Biomass = 11.776g</td>
</tr>
<tr>
<td>0.9983 mol O_2 = 31.9456g</td>
<td>0.52106 mol CO_2 = 22.93g</td>
</tr>
<tr>
<td>0.095793 mol NH_4OH = 3.35265g</td>
<td>1.811 mol H_2O = 32.598g</td>
</tr>
<tr>
<td>Total mass in = 67.2984g</td>
<td>Total mass out = 67.305g</td>
</tr>
</tbody>
</table>

**TABLE 4:** Mass balance for Fed-batch mode-III

<table>
<thead>
<tr>
<th>Mass in</th>
<th>Mass out</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mol Methanol = 32.0000g</td>
<td>0.4789 mol Biomass = 11.776g</td>
</tr>
<tr>
<td>0.99834 mol O_2 = 31.9469g</td>
<td>0.5211 mol CO_2 = 22.93g</td>
</tr>
<tr>
<td>0.09579 mol NH_4OH = 3.35265g</td>
<td>1.81085 mol H_2O = 32.5953g</td>
</tr>
<tr>
<td>Total mass in = 67.29955g</td>
<td>Total mass out = 67.3017g</td>
</tr>
</tbody>
</table>

![Figure 3: Bioreactor configuration with the calculated design parameters](image3)
Bioreactor design for the production of recombinant human therapeutic protein

Full Paper

TABLE 5: Mass balance for Fed-batch mode-IV

<table>
<thead>
<tr>
<th>Mass in</th>
<th>Mass out</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mol Methanol = 32,000g</td>
<td>0.4789 mol Biomass = 11.776g</td>
</tr>
<tr>
<td>0.99834 mol O₂ = 31.9469g</td>
<td>0.5211 mol CO₂ = 22.93g</td>
</tr>
<tr>
<td>0.095785 mol NH₂OH = 3.3525g</td>
<td>1.81083 mol H₂O = 32.595g</td>
</tr>
<tr>
<td>1.57352×10⁻⁵ mol Protein = 0.000349g</td>
<td></td>
</tr>
<tr>
<td>Total mass in = 67.2949g</td>
<td>Total mass out = 67.30135g</td>
</tr>
</tbody>
</table>

TABLE 6: Overall design calculation results

<table>
<thead>
<tr>
<th>S.No</th>
<th>Design parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hₐ</td>
<td>4300 mm</td>
</tr>
<tr>
<td>2</td>
<td>Hₐ*</td>
<td>5770 mm</td>
</tr>
<tr>
<td>3</td>
<td>Hₐ**</td>
<td>6350 mm</td>
</tr>
<tr>
<td>4</td>
<td>Dₚ</td>
<td>3300 mm</td>
</tr>
<tr>
<td>5</td>
<td>Dₗ</td>
<td>1300 mm</td>
</tr>
<tr>
<td>6</td>
<td>N</td>
<td>3.8 rps</td>
</tr>
<tr>
<td>7</td>
<td>Nᵢ</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>Vₑ</td>
<td>34.4 mm s⁻¹</td>
</tr>
<tr>
<td>9</td>
<td>Qₑ</td>
<td>0.2942 m³ s⁻¹</td>
</tr>
<tr>
<td>10</td>
<td>vvm</td>
<td>0.4843</td>
</tr>
<tr>
<td>11</td>
<td>Q</td>
<td>2606.864×10⁻¹ J s⁻¹</td>
</tr>
<tr>
<td>12</td>
<td>m</td>
<td>41.5 kg water s⁻¹</td>
</tr>
<tr>
<td>13</td>
<td>tₚ</td>
<td>0.94 mm</td>
</tr>
<tr>
<td>14</td>
<td>tₗ</td>
<td>0.94 mm</td>
</tr>
<tr>
<td>15</td>
<td>Rₚ</td>
<td>3300 mm</td>
</tr>
<tr>
<td>16</td>
<td>Rₗ</td>
<td>198 mm</td>
</tr>
<tr>
<td>17</td>
<td>Dₙ</td>
<td>77.60 mm</td>
</tr>
<tr>
<td>18</td>
<td>Nₚ</td>
<td>5 turns</td>
</tr>
<tr>
<td>19</td>
<td>J (Dₚ/12)</td>
<td>275 mm</td>
</tr>
<tr>
<td>20</td>
<td>E (Dₚ/3)</td>
<td>1100 mm</td>
</tr>
<tr>
<td>21</td>
<td>W (Dₚ/5)</td>
<td>260 mm</td>
</tr>
<tr>
<td>22</td>
<td>L (Dₚ/4)</td>
<td>325 mm</td>
</tr>
</tbody>
</table>

Mass in Mass out

1 mol Methanol = 32,000g
0.4789 mol Biomass = 11.776g
0.99834 mol O₂ = 31.9469g
0.5211 mol CO₂ = 22.93g
0.095785 mol NH₂OH = 3.3525g
1.81083 mol H₂O = 32.595g
1.57352×10⁻⁵ mol Protein = 0.000349g

Total mass in = 67.2949g
Total mass out = 67.30135g

kₐ was calculated as 0.080 sec⁻¹. The design calculations were performed with various trial and errors in order to obtain the same kₐ a value which was one of the criteria for checking the considered design parameters were correct in this theoretical design procedure. Once, both the kₐ values were nearly equal, the assumed design parameters were considered to fit best for the bioreactor and then mechanical design was carried out to evaluate the mechanical design parameters with different relationships available from literature. All the assumed parameters and calculated values were tabulated in TABLE 6.

Conclusion

The bioreactor design calculations used in this work is easy to learn and offers a quicker way for preliminary calculations of the bioreactor design. This method also enables the designers to scale up and optimize the bioprocess. By using this method the designer also escapes the costly simulation software license. Moreover, this work was completely carried out with plenty of assumptions since the design is theoretically done. So, if the exact experimental values are available for a particular protein or enzyme production using a particular microbe, then definitely the approach used in this work will benefit the designers to come up with exact design parameters.

References