Optimization of Design Variables and Operational Parameters for Maximizing Annualized Profit of Industrial L-lysine Fermentation

Clay Swackhamer
Rishi Dantuluri
Shivani Patel
Casey Hall

Biological Engineering
Spring 2015
BE 469

Table of Contents

Executive Summary

A multi-design variable optimization has been conducted for industrial scale batch fermentation with the goal of maximizing total yearly profit. The optimization model has been applied to industrial fermentations of L-lysine, but future work could extend it to vanillin, tetracycline, or other industrially significant biomolecules.

The model is limited by several constraints, such as that the fermentation considered must be by batch. Labor, administration, and maintenance costs have been neglected due to highly variable parameters needed for accurately considering for these costs. Processing costs have also not been examined, as these are not strictly part of the primary fermentation. In essence, the control volume for this optimization is the fermenter itself and thus ancillary systems such as facility maintenance, processing, and administrative overhead have not been analyzed. For known information about media, cellular growth, and product formation, the model can be used to determine optimal temperature, agitation, dissolved oxygen concentration, and volume per reactor.

The results of optimizing the system are summarized in table 1.

Table 1: Optimal design variables

Agitation	Ni	81.6	rpm
Volume per reactor	Vtotal	1000000	L
Temperature	T	30.18	C
Dissolved O2 concentration	DO	0.25	fraction of saturation from 0-1

These values fall into acceptable ranges. The model is tested by fixing certain design variables and re-optimizing. In all cases, the expected effects of changing the design variables are found to be calculated by the model, and the new optimal designs are also reasonable.

Problem Statement

Industrial fermentations are important because they are a huge producer of value added products. Microorganisms are used in such systems to produce products that used in many different applications in the food industry and chemical industry. Fermentation of L-lysine has been chosen due to its large global scale, as well as application to growing industries including animal supplementation and human dietary supplementation. By increasing amino acid absorption through supplementation of L-lysine, the total nitrogen content in manure can be decreased, reducing environmental impact of agricultural operations. This is an important application of L-lysine as sustainable agricultural practices are increasingly adopted. (Kerr et al., 2003).

Use of chemical synthesis to create lysine creates a racemic mixture of both D- and L-lysine. Even though primary production via this synthetic pathway is typically more economical than biological methods, downstream processing is very difficult due to the problem of needing to separate the two stereoisomers, L-lysine and D-lysine (Gorton, 1963). Since all known life can only metabolize L amino acids, D-Lysine produced in this synthetic process is a waste product (Brignole and McDowell, 2001), and industrial processes to either convert it to L-Lysine or remove it from racemic mixtures on a commercial scale are not attempted.

In order to achieve a production of L-lysine which results in a product that is biologically available, a biological step must be introduced in production, preventing the appearance of the D-Lysine stereoisomer (Anastassiadis, 2007). Use of a microbial fermentation absolves the need for separating the two optically active forms (Anastassiadis, 2007), and also presents an opportunity to build on a process that has been employed since the 1960's to produce L-Lysine (Toride, 2002). In a bacterial fermentation, a microorganism is used which is capable of synthesizing the desired product, an amino acid, from nutrient sources which are inexpensive, such as simple sources of carbon and nitrogen.

On of the main components in a biological production process for L-lysine is the reactor where the fermentation occurs. Some trade-offs in reactor design include reactor volume and agitation speed. The

volume of the reactor increases the amount of product that can be produced in a single batch, as well as the salvage costs of the reactor, but also increases the capital costs as well as heating and aeration costs. Agitation speed is a trade off because it can increase the dissolved oxygen concentration (DOC) in the medium, which increases the yield, but also requires more energy. Both agitation speed and reactor volume cannot be increased or decreased without bounds, as there are reasonable physical limitations that must be met in any industrial process. Temperature is another trade off, because operating at higher temperature drives up heating costs, but operating at temperatures lower than the ideal temperature for any particular fermentation will result in sub-optimal yield due to poor growth and metabolism by the microorganism.

Model Formulation

Design Variables

The design variables that were examined are volume of reactor (L), agitation rate (rpm), temperature (°C), and dissolved oxygen concentration (DOC, %DOCmax). The model was applied to industrial scale L-lysine fermentation. Each of the above design variables was changed manually, and the effects were observed on the intermediate cost terms in order to ensure that the model was functioning properly. The design variables include a reactor design parameter (volume) as well as several operational parameters. The design variables are summarized in table (2).

Table (2): Design Variables

Design Variable	**Unit**
Volume of reactor	L
Agitation	rpm
Temperature	°C
Dissolved Oxygen Concentration	%DOCmax

Objective Function: Maximize Total Annual Profit

In order to develop an optimization scheme for maximizing total annual profit, a total yearly profit equation was developed. This equation uses several broad categories of revenue and costs, and is presented in equation (1).

Equation (1)

$$Profit\left(\frac{\$}{yr}\right) = revenue\ from\ product\ produced + annualized\ ajusted\ salvaje\ value - annualized\ capital\ costs - operating\ costs$$

Each of the four terms in the overall profit equation was broken down further, into its own category of equations. First, the revenue from produced product is modeled by equation (2).

Revenue from product produced

Revenue from product that is produced in the fermentation system depends on several factors. Predictably, increases in product value, product concentration in the medium, operational hours per year,

and volume per reactor all result in increased revenue. Increases in idle time and time per batch both result in lower revenue.

Equation (2)

$$Revenue\ from\ product\ produced = N_r * \left(\frac{t_y}{t_f + t_{cl}}\right) * V_f * P_{tf} * C_p$$

(Modified from Kookos, 2004)

Where:

- t_y = operational hours per year = 8,000 (hr)
- t_f = batch time (hr)
- t_{cl} = cleaning and idle time = 0.05* t_f (hr)
- C_p = price of product ($/g) = 0.0012 ($/g) (Alibaba.com)
- V_f = working volume = 0.8*V_{total} (L)
- P_{tf} = final product concentration (g L-lysine /L)

Operational hours per year is estimated by assuming the plant will function on a 24 hour schedule for 11 months each year. Batch time is calculated by adding the times for lag phase, logarithmic phase, stationary phase, and idle time for the process.

Equation (3)

$$Total\ batch\ time = \ t_f = time_{lag} + \ time_{log} + \ time_{stationary}$$

Log phase time is calculated by integrating the first order kinetic equation which describes logarithmic microbial growth, and is shown here in equation (4).

Equation (4)

$$\frac{dx}{dt} = \ \mu x$$

$$\frac{dx}{x} = \ \mu dt$$

$$\int_{x_o}^{x} \frac{dx}{x} = \int_{0}^{t} \mu dt$$

$$\ln(x) - \ln(x_o) = \mu t$$

$$\ln\left(\frac{x}{x_o}\right) * \frac{1}{\mu} = time_{log}$$

For fermenters with this microorganism grown to saturation, the final biomass value is approximately 20 g/L (Heinzle, et al. 2006). This is the value of x, but to evaluate $time_{log}$, a value for initial biomass is also needed. This is assumed to be one percent of the final biomass concentration, which will be introduced via an inoculum.

Equation (5)

$$Initial\ concentation_{reactor} = \left(20\frac{g}{L}\right) * (0.01)$$

$$Initial\ concentation_{reactor} = x_o = 0.2\ \frac{g}{L}$$

This is substituted into the equation for log phase time, but in order to calculate the time, a value for μ, the specific growth rate, must be found. The specific growth rate is calculated from an equation where it has been modeled as a function of several parameters which depend on DOC.

Equation (6)

$$\mu = k * S^n$$

(Yao, et al. 2001)

Where:

k= experimentally derived constant

μ = specific growth rate (1/min)

n = experimentally derived constant

The constants k and n are found by regression equations dependent on DOC, where DOC is expressed as a percentage of DOC max. The maximum DOC is found for the same medium, at 30°C. Even though the solubility of oxygen changes with temperature, these variations are minor and assumed to be negligible. Modeling all of this as a function of the two design variables T and DOC would be a potential for improvement in the model.

Equation (7)

$$k = 1 * 10^{-7} * 1.2986 * e^{0.47485 * DOC}$$

Equation (8)

$$n = -0.0038226 * DOC^2 + 0.01326 * DOC + 2.0418$$

Example values are shown for a reasonable DOC and substrate concentration, S.

Table (3) k, n and μ at DO = 0.25

DO	**k**	**n**	**S (g glucose/L)**	**μ (1/min)**
0.25	1.46228E-07	2.0448761	50	0.247

Using these values, the equation for log phase time can be solved.

Equation (9)

$$\ln\left(\frac{x}{x_o}\right) * \frac{1}{\mu} = time_{log}$$

$$\ln\left(\frac{20g/L}{0.2g/L}\right) * \frac{1}{0.247/hr} = time_{log}$$

$$time_{log} = 18.6\ hr$$

Next, the other terms in the total time equation are determined. From the graph below, the necessary stationary time can be estimated at 35 hr. The lag time can likewise be estimated at 15 hr.

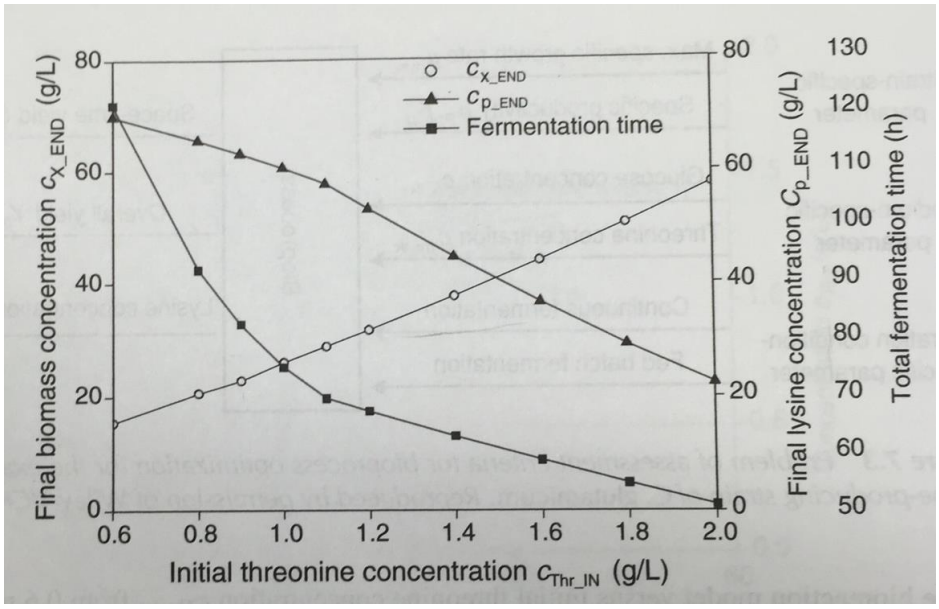

Figure (1): Graph of L-Lysine Fermentation (Shuler, L. Michael. Kargi, Fikret. 2002)

These values are then substituted back into equation (10) for batch time, which is evaluated.

Equation (10)

$$Total\ batch = \ t_f = time_{lag} + \ time_{log} + \ time_{stationary}$$

$$t_f = 15\ hr + \ 18.6\ hr + \ 35\ hr$$

$$t_f = 68.6\ hr$$

Idle time is calculated by assuming direct proportionality to overall batch time. This includes harvesting, cleaning, refilling, and sterilization.

Equation (11)

$$T_{idle\ and\ cleaning} = t_{cl} = 0.05 * t_f$$

This time is calculated for the above example.

Equation (12)

$$t_{cl} = 0.05 * 68.6$$

$$t_{cl} = 3.43\ hr$$

The yield of product is likewise determined by several intermediate calculations. First, the concentration of product in the reactor (at the end of the batch) can be modeled as a function of total substrate in the medium, and the yield of product relative to substrate.

Equation (13)

$$P_{tf} = Y\frac{P}{s}\left(\frac{g\ L - lysine}{g\ glucose}\right) * S(g\ glucose)$$

Where:

- $Y\frac{P}{s}$ = yield of product relative to substrate (g product produced / g substrate used)
- S = Initial substrate concentration = (150 g glucose/L)

This model assumes total substrate exhaustion, which is not unreasonable for batch fermentations operated for long periods of time in which secondary metabolites are produced, which is the case for L-lysine. The yield factor is dependent on the dissolved oxygen concentration (DOC) of the media, which is defined as the percentage of the saturation oxygen concentration of the same medium at 30°C. The yield is also dependent on the temperature at which the fermentation is conducted, because deviations from the experimentally derived optimal temperature for L-lysine fermentation by *C. glutamicum* are known to result in sub-optimal yields (Shah, et al, 2002), (Razak and Viswanath, 2015). In the case of this fermentation, a medium has been selected that has already been optimized for use in amino acid production by *C. glutamicum*, which contains 150 g/L substrate. These results are shown in table 4.

Table 4: Fermentation medium composition

Fermentation media				
$CaCl_2{\cdot}2H_2O$	$(NH_4)_2SO_4$	$MgSO_4{\cdot}7H_2O$	NaCl	$MnSO_4{\cdot}H_2O$
1.0	**20.0**	0.4	0.05	0.0076
$FeSO_4{\cdot}7H_2O$	KH_2PO_4	K_2HPO_4	Urea	Yeast Extract
0.01	0.5	0.5	2.0	1.0
Peptone	D-glucose	Thiamine·HCL	D-biotin	**L-serine**
1.0	**150.0**	0.0002	0.0005	**0.0001**

(Yao, et. At, 2001)

Table (5): Yield factor based on DOT

DOT	$Y\frac{P}{s}\left(\frac{g\ product}{g\ substrate}\right)$	$P_{tf}\ \left(\frac{g\ product}{L}\right)$
20%	0.654	98.1
10%	0.454	68.1
5%	0.359	53.9
2%	0.342	51.3

The values of YP/S is calculated from DO based on a regression equation from Powell.

Equation (14)

$$Y\frac{P}{S} = 0.20089 * e^{(0.049438*DO)} + 0.11591$$

This contributes to a trade off in the design, because DOT is dependent on the aeration and agitation, which drive operational costs, yet also contributes to high yield, raising the revenue from product that is produced. In order to quantify this relationship, the actual concentration of oxygen in the medium must be found from the value of DOT.

The dissolved oxygen tension is expressed as a percentage of the saturated oxygen concentration at the conditions of the fermentation. Because of this, the saturated oxygen concentration must be found in order to determine which true concentration of oxygen in the medium corresponds to each value of DOT. This requires calculations of oxygen transport, which are dependent on the thermodynamic equilibrium of oxygen gas with the aqueous solution in the bioreactor. The equilibrium concentration of dissolved oxygen at atmospheric pressure and 25°C, for a moderate salt content which approximates the fermentation media, 6.8 mg O_2/L (Demirci, 2015). This value is taken from a standard table.

$$C^*_{L\,std} = 6.8\ \frac{mg\ O_2}{L}$$

Next, the partial pressure of water vapor in the bioreactor is calculated from the Antoine equation, for a temperature of 25°C.

Equation (15)

$$log_{10}(Partial\ pressure\ water\ vapor) = \ 5.4 - \frac{1839}{(T\ in\ °K - 31.7)}$$

$$log_{10}(Partial\ pressure\ water\ vapor) = \ 5.4 - \frac{1839}{(298 - 31.7)}$$

$$Partial\ pressure\ water\ vapor = 22.5\ mm\ Hg$$

In order to calculate the actual concentration of dissolved oxygen in the reactor, the total pressure in the reactor must be determined. The absolute pressure in the reactor is calculated by approximating the gage pressure as 5 psi, (which is largely dependent on the type of filter used on the air vent (Demirci, 2015)) and adding the atmospheric pressure at sea level.

Equation (16)

$$P_{abs} = P_{gage} + P_{atm}\ (17)$$

$$P_{abs} = 5psi * \frac{51.7\ mm\ Hg}{psi} + 760\ mm\ Hg$$

$$P_{abs} = 1018\ mm\ Hg$$

Now that the equilibrium concentration of dissolved oxygen in the bioreactor, the partial pressure of water vapor, and the pressure in the reactor are known, the actual equilibrium concentration of dissolved oxygen can be calculated from equation (17)(Demirci, 2015) .

Equation (17)

$$C^*_{L\,calculated} = C^*_{L\,std} * \frac{(P_b - P_v)}{(P_{atm} - P_v)}$$

Where:

P_b = absolute pressure in the reactor = 1018 mm Hg

P_v = Partial pressure of water vapor = 22.5 mm Hg

P_{atm} = Atmospheric pressure =760 mm Hg

$C^*_{L\,std}$ = equilibrium concentration of dissolved oxygen at atmospheric pressure and 25°C = 6.8 $\frac{mg\ O_2}{L}$

$$C^*_{L\,calculated} = 9.2\ \frac{mg\ O_2}{L}$$

Oxygen uptake is the oxygen taken from liquid phase, dissolved in the culture medium, and used by cells for aerobic processes. This must be satisfied in order to prevent the cells from undergoing less efficient metabolism and incurring both decreased growth and decreased production.

It is assumed that oxygen uptake rate (OUR) is satisfied by oxygen transport in the reactor, which is the ability of the system to move oxygen from the gaseous phase into the dissovled phase in the liquid. This is important because overtransport is not necessary for the cells, and will only drive up aeration and agitation costs, whereas not satisfying the demand will lead to poor production. It is a constraint in the model that OUR = OTR for any design that is produced.

Next, the mass transfer coefficient Kla must be calculated by the following equation.

Equation (18)

$$Kla = a * \left(\frac{Pg * 1000}{Vworking}\right)^{b} * Us^{c}$$

In this equation, a, b, and c are empirically derived constants, which are taken from Powell as 0.02, 0.6,0.6, but are modified to 0.05, 0.8,0.8 for this model to get more reasonable results. The variable Pg is the impeller power, which is calculated in kW from equation 19.

Equation (19)

$$Pg = impeller\ power = Pn * rho * \left(\frac{Ni}{60}\right)^{3} * dimp^{5}/1000$$

Where:

Pg = impeller power (kW)

Dimp = impeller diameter (m) = 0.1-0.3*Dinner

Rho = density of medium = 1074 kg/m^3

Pn = power number = 5 (for rushton type impeller)

The cost of agitation is modeled from the impeller power with equation 20.

Equation (20)

$$Cost\ of\ agitation = Pg * Ce * ty$$

Where:

Pg =impeller power (kW)

Ce = cost of energy = 0.1 $/kW*hr

Ty = operational hours per year = 8,000 hr

Revenue from adjusted annualized salvage value

The salvage value is the monetary gain that is experienced when the equipment is sold after its functional lifespan is reached. Revenue from salvage is inherently difficult to model because it is based on the value of equipment at a future date. In order to model it, the lifespan of equipment, depreciation rate, and inflation rates must all be estimated. This economic information allows a future one-time value to be calculated, and using engineering economics this can be converted into an annualized reward.

In order to solve for the salvage cost, the goal is to first solve equations 3 and 4 for D_i and h of the reactor in terms of volume of the steel. We assumed the thickness of the steel, th to be 0.1 m. The working volume, $V_{working}$ can be calculated using equation 5. There are two equations and two unknowns, since $V_{working}$ is known. Next, V_{steel} is solved as a function of $V_{working}$ (as shown in Eq 6). The total volume V_{total}, is the $V_{working}$ times the head space times a factor of 0.2 (as shown in Eq 7). Then, the salvage cost can be calculated by multiplying the total volume of the steel used (from equation 8) times the price of the steel.

Equation (21)

$$\frac{D_i}{h} = \frac{1}{3}$$

After rearranging the above equation we get,

$$D_i = \frac{h}{3}$$

Equation (22)

$$D_o = D_i + 2 * th$$

Equation (23)

$$h = \left(\frac{\frac{V_{total}}{1000}}{0.10472}\right)^{\frac{1}{3}}$$

Equation (24)

$$V_{working} = \frac{\pi}{4} {D_i}^2 * h$$

Equation (25)

$$V_{steel} = \frac{\pi}{4} * (D_o - D_i)^2 * h + 2 * \frac{\pi}{4} {D_o}^2 * th$$

Equation (26)

$$V_{Total} = V_{working} + 0.2 * V_{working}$$

Equation (27)

$$Salvage\ Value = \frac{A}{P} * (V_{Steel} * C_{steel})$$

Where:

- D_o = Outer Diameter in m
- D_i = Inner Diameter in m
- h = height of tank in m
- th = thickness = 0.1m
- V_{steel} = Volume of the steel used in m^3
- C_{steel} = Cost of steel = 13280 $/m^3$

A over P is calculated in equation 28.

Equation (28)

$$A/P = \frac{i(i+1)^N}{(1+i)^N - 1}$$ i=interest rate= 8% ; N=number of years=20.

The only variable changing here is $V_{working}$, the h and D will be calculated using Equations 3 and 4.

The annualized income from salvage value is calculated by estimating the current salvage value, converting this to a future value using an F over P factor, then annualizing this result with an A over F factor. The equations for F over P and A over F are shown below.

Source: http://www-old.me.gatech.edu/

F over P = (1+i)^n = 1.17

A over F = i/((1+i)^n-1) = 0.470

Where:

i = time value of money = 0.08

n = service life = 20 yrs

Operating costs

The operating costs of the reactor system are difficult to model because it is difficult to state for certain which operational and design parameters will influence operational costs, and how much effect they may have on costs not intrinsic to the reactor. For example, higher agitation rates can be predicted to increase energy costs, but may result in more frequent and expensive maintenance for equipment, more expensive equipment to begin with, reduced salvage value for equipment, and possibly even higher labor costs if more managers are required to operate systems running closer to their operational capabilities. In the context of this model, where a control volume has essentially been drawn around the reactor itself, outside effects of changing operational parameters on labor costs have been ignored, as have effects on maintenance. The only two terms in the operational costs equation that remain are energy costs and materials costs. Intuitively, more powerful equipment results in higher energy costs, and larger reactors holding more medium requires more inputs of materials. These costs are modeled in equation 29.

Equation (29)

$$Operating\ costs = Energy\ costs + Materials\ costs$$

Materials costs are found by equation (30).

Equation (30)

$$Materials\ costs = (C_s * V_f * + X_0 * C_x * V_f) * N_{batch} * N_r\ (From\ Kookos\ eq. 18)$$

Where:

- C_s = cost of medium ($/L)
- V_f = working volume = 0.8*V_{total} (L)
- X_0 = initial concentration of biomass (g biomass/L)
- C_x = cost of inoculum ($/g biomass)
- N_{batch} = number of batches per year for one reactor
- N_r = number of reactors running in parallel

Energy costs are found by equation (31).

Equation (31)

$$Energy\ costs = cost\ agication + heating\ cost$$

Equation (32)

$$Cost\ agitation = P_g * C_r * t_y * N_r\ (Powell\ eq. 34)$$

Equation (33)

$$cost\ of\ heating = \ (\rho_i * Q_i * C_P * (T - T_i)) * C_p)\ (Yetilmezsoy\ eq. 16)$$

Where:

- W_c = aerator power (kW)
- P_g = agitator power (kW)
- C_p = cost of energy ($/kW*hr) = 0.1 $/kWhr
- t_y = operational hours per year = 8,000 (hr)
- N_r = number of reactors running in parallel

- ρ_i = density of medium (kg/m^3)
- Q_i = influent flow rate (m^3/day)
- C_p = specific heat of the medium (J/kg/K)
- T = operating tem
- perature (°C)
- T_i = temperature of medium (°C)

Annualized Capital Costs

Capital costs are one-time costs that are incurred when the operation is first set up. They may be adjusted using engineering economics and annualized. Capital costs for equipment that is not part of the reactor unit is not considered. Minor equipment is not considered, even if it makes up part of the reactor system. This includes units such as pH probes, anti-foaming units, condensers, filters, etc. The annualized capital costs of reactors and equipment can be calculated using equation 34.

Equation (34)

$$Annualized\ Capitcal\ costs = C_{capannual}(\frac{\$}{yr}) = \frac{A}{P} * (cost\ reactors + cost\ agitation)$$

Where A over P is found in equation 35.

Equation (35)

$$\text{A/p} = \frac{i(i+1)^N}{(1+i)^N - 1}$$

I – Interest rate = 8 %

N- Number of years = 20

Cost of the reactors is found in equation 36.

Equation (36)

$$Cost\ \ reactors = C_{react}\left(\frac{\$}{yr}\right) = 40{,}000 * \left(\frac{V_{total}}{0.5}\right)^{0.6} * N_r\ (Powell\ eq. 32)$$

Where:

- V_{total} - Total volume per reactor
- N_r - number of reactors running in parallel = 1

Cost of the equipment is limited to agitator (mixer). For agitator this is mainly an electric motor and speed differential system, as well as a shaft and impeller. The cost of the agitator can be calculated using equation 37.

Equation (37)

$$Cost\ agitation = C_{mix}\left(\frac{\$}{yr}\right) = 2.5 * \left(847.65 * \left(\frac{P_g}{1000}\right) + 8423.6\right)(Powell\ eq. 20)$$

Where

P_g – Agitation power in kW.

Constraints:

All of the capital cost equations are valid only on certain cost intervals, which are specified by Powell.

Other constraints are summarized in table (6) :

Constraints					**unit**
10	<=	Ni	<=	150	rpm
10000	<=	Vtotal	<=	1.00E+06	L
27	<=	T	<=	35	C

0.1	<=	DO	<=	0.25	fraction of saturation from 0-1
ourmax	<=	otr			

These constraints are based on knowledge of the general fermentation process. First, to ensure turbulent conditions and adequate mixing, the rpms of the impeller must be reasonable. The volume of the reactor cannot be too large. Dissolved oxygen must be high enough to allow microbial growth, but not too high, as the regression equations for Yield as a function of DO are not valid past 25% saturation. Temperature is used to penalize yields for deviating from the experimentally derived optimal temperature, which is taken from an average of three data sets for the same fermentation, albeit on a benchtop scale. These equations are only valid on the interval that is used to constrain temperature. Lastly. OTR must be greater than or equal to OUR in order to allow the microorganisms to grow.

Model Implementation

Once all four terms in the overall costs equation are calculable as functions of the design variables, the model can be optimized in Excel. This has been accomplished using the generalized reduced gradient (GRG) solver, as all functions are continuous with no table lookups, integer constraints, or piecewise functions. All components of the objective function are continuous and differentiable across their constrained domains, and because of this, the GRG solver is predicted to work. The GRG algorithm is known to be less time consuming than the evolutionary solver, providing another incentive to use it for early implementation.

The model is solved using the constraints on design variables that make physical sense with the model. Variables temperature and DOC are constrained across rather narrow ranges because other components of the model are calculated based on these variables, and regression equations are available, but their domains cannot be exceeded.

In the initial runs of solver, the impeller speed is calculated at 81.6 rpm, which falls into the middle of the allowable range (10-150 rpm). This must satisfy the constraint that oxygen uptake rate cannot exceed oxygen transfer rate, and according to the equations, 81.6 rpm on the impeller will provide sufficient mass transfer for the necessary aeration to occur. The equation for Kla was modified using different empirical constants a,b, and c.

Optimal DOC is found at 0.25, which means 25% DOCmax at 30°C. Insufficient dissolved oxygen results in a lower yield, based on regression equations for yield as a function of DOC. This value of DOC is known to be reasonable based on the references that were examined. The total volume is driven to the maximum allowable value of one million liters, and this is predictable, because if the reactor is profitable per unit volume, it makes sense that the model will increase volume until it reaches its upper constrained value in order to maximize profit.

The temperature provides an interesting case, as it is optimized in between the lower bound of 27°C and the upper bound of 32°C. It is noticed that minor deviations from the optimal temperature result in large losses in product revenue, and this is also expected based on the results of other researchers. The specifics of the L-lysine biosynthetic pathway as well as the conditions for best growth of *C. glutamicum* both stipulate that temperature be tightly controlled.

This run of solver yields optimal values which are summarized in table (7).

Table (7): Optimal values of design variables

Agitation	Ni	81.6	rpm
Volume per reactor	Vtotal	1000000	L
Temperature	T	30.18	C
Dissolved O2 concentration	DO	0.25	fraction of saturation from 0-1

In preliminary solver runs, Kla was greatly overestimated, resulting in very low optimal values of rpms being calculated. The most noticeable change once this was rectified is that the number of rpms has been increased. This is necessary to ensure that oxygen transfer can meet the oxygen uptake demand. The values that are calculated are all reasonable, based on prior knowledge of large scale L-lysine fermentations. A rudimentary cost breakdown is presented in table (8) based on the optimum design variable values.

Table (8). Profit breakdown at optimal values of design variables

Costs summary:		
Profit	$1,037,918.30	$/yr
Revenue from product produced	$4,967,447.25	$/yr
Annualized Salvage Value	$61,868.33	$/yr
cost of agitation	$465,118.38	$/yr
cost of heating	$298,229.60	$/yr
Annualized Capital Costs	$ 391,896.85	$/yr

Using this simple breakdown, a basic sensitivity analysis can be conducted. First, temperature is decreased to 29 °C, and removed from the alterable design variables. This is a modest change, but one that is predicted to greatly lower yield, and thus revenue. It is predicted to also lower the heating costs. The predicted effects are all noticed, as heating costs and revenue from product have both decreased, while all other design variables remain at their optimal values. The results are displayed in table 9.

Table 9: Costs for fixed temperature of 29°C

Costs summary:		
Profit	$ 901,878.78	$/yr
Revenue from product produced	$4,817,390.12	$/yr
Annualized Salvage Value	$61,868.3	$/yr
cost of agitation	$465,118.3	$/yr

cost of heating	$284,211.98	$/yr
Annualized Capital Costs	$391,896.85	$/yr

The next variable that is explored is DO. This variable can only be changed within the limits that allow it to still be used to calculate $Y_{P/S}$, based on regression equations that are only valid on the interval from 0.1- 0.25, as well as with the constraint that oxygen transfer equals oxygen uptake. To determine the absolute highest value of DO that can be achieved using the modeled systems capability for oxygen transfer, as simple "what if" analysis is conducted using goal seek. It is recognized that the highest value of DO will only be achievable if rpms are maximized, and so rpm is set to 150, the highest allowable value, and removed from the design variables. Then, OTR is set to the maximum value of OUR, because maximum uptake will required maximum transfer, while changing the cell that houses DO. This will yield the theoretically highest achievable value of DO by the system. This is based on equation (38).

Equation (38)

$$OTR = OUR\ max = Kla * (Cl^* - CL)$$

When OUR is equal to OUR max, the highest demands for oxygen transfer must be met. Raising DO raises CL, and reduces the magnitude of the quantity $(Cl^* - CL)$. This means that the highest possible Kla must be achieved, which corresponds to the highest rpms that are allowable. Goal seek is set up so that rms are maximized, giving the highest possible value of Kla, and OTR is constrained to equal OUR max, while changing DO. Thus, the value of DO which is the maximum possible allowable value will be calculated, because this is the value which allows the previous conditions to be satisfied.

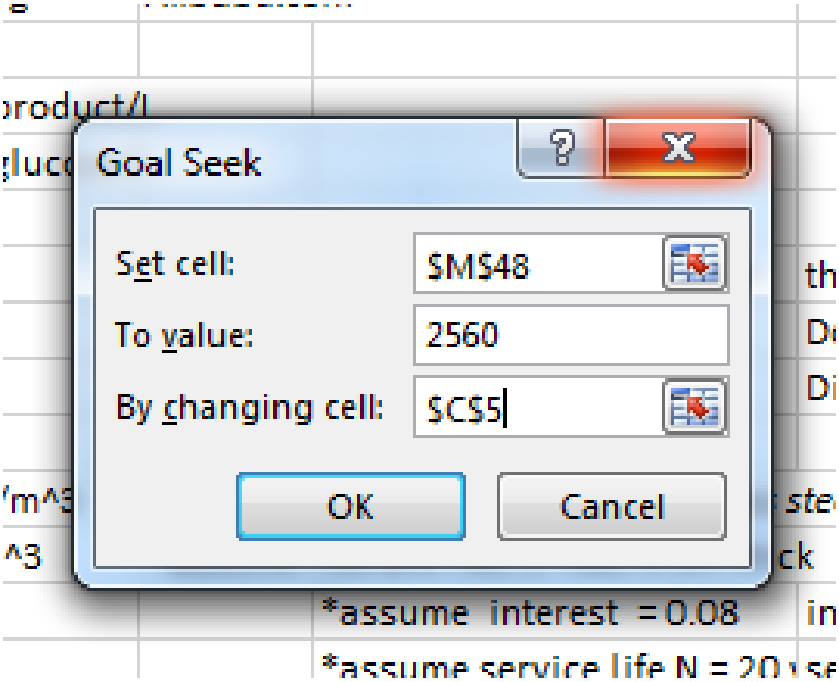

Figure (2) Goal seek setup

The maximum uptake is 2560 mg O2/ L*hr. The result is that DO max is found to be 0.79. Since this is outside the allowable range based on the regression equations for DO (0.1-0.25), it is assumed that the highest value is 0.25. This solving analysis was conducted to determine whether the maximum value of DO that falls into the domain of the regression equations is actually achievable by the system, and it is found to be achievable.

Next, DO is set to 0.25 and the model is solved again, yielding new optimal design variables as well as new costs. The new optimal values of design variables are displayed in table 10.

Table 10: Optimal values of design variables when DO fixed to 0.25

Agitation	Ni	88.0	rpm
Volume per reactor	Vtotal	1000000	L
Temperature	T	30.18	C
Dissolved O2 concentration	DO	0.25	fraction of saturation from 0-1

The only change from the previous design variables is that DO is artificially kept to 0.25 (was not included as a design variable in the solving step), and that agitation must increase in order

to satisfy the new need for transfer. When DO is increased, the term (CL*-CL) decreases in the equation for OTR.

$$OTR = Kla * (Cl^* - CL)$$

This means that a higher KLa is needed at higher DO, which makes sense intuitively, and also requires higher aeration power, which is predicted to drive higher aeration costs. These new costs are presented in table 11.

Table 11: Costs summary for design where DO fixed to 0.25

Costs summary:		
Profit	$976,954.81	$/yr
Revenue from product produced	$5,104,056.50	$/yr
Annualized Salvage Value	$61,818.36	$/yr
cost of agitation	$584,168.33	$/yr
cost of heating	$304,992.31	$/yr
Annualized Capital Costs	$391,928.97	$/yr

As expected, agitation costs have increased, as have revenue totals. However, the increased costs from agitation outweigh the benefits of increasing yield, which is reflected in the overall profit statement.

Conclusions and Recommendations

In conclusion, a model has been developed to successfully calculate the design variables which could be used to design and operate an industrial scale L-lysine fermentation operation. The model has been validated only by artificially changing design variables, re-solving, and analyzing the new costs, however, this analysis seems to show that the model is functioning as expected. Several

interesting results are reached. First, the optimal temperature for L-Lysine fermentation may not be 30 °C, instead a value of 30.18°C is predicted. This may be beyond the accuracy of actual temperature controls for such a large scale system, but could merit the installation of more accurate controls. The model predicts higher yield at higher DO, but also higher aeration costs, which outstrip the increase in yield. It also predicts lower yield, but also lower heating costs at lower temperatures, but in this case the lost yields win out and the optimal temperature is found to be above 30°C.

The recommended design for this system is reiterated in table 12.

Table 12: Optimal values of design variables reiterated

Agitation	Ni	81.6	rpm
Volume per reactor	Vtotal	1000000	L
Temperature	T	30.18	C
Dissolved O2 concentration	DO	0.1	fraction of saturation from 0-1

Possible goals to improve the model implementation include applying it to other industrial batch fermentations, including costs that were not considered, such as processing costs, which could theoretically be found to depend on design variables (particularly the volume of the reactor), as well as including the costs of aeration compressors (this equation was in one of the references but very confusing). The model could be validated experimentally, but the scale involved makes this difficult. The model could be run using a sensitivity analysis, and this would be useful for operators making decisions on allowable operational parameter tolerances.

Another feasibility check which could be useful to the model would be to experimentally validate the costs of heating the medium. This model assumes that the culture produces no heat through cellular processes, but this is not actually the case, as all metabolic processes involve the

release of heat, and aerobic metabolic schemes are known to be particularly exothermic. This could mean that the culture is essentially self-heating, although the feasibility of engineering such as system into batch production is not intuitive. It is even possible that the medium would need to be cooled to keep temperature from rising past the optimal value.

Another limitation of the model is that the maximum solubility of oxygen in the culture medium is not calculated as a function of temperature. This means that the values of DO that are used as design variables always correspond to percentage of oxygen solubility at 30°C. This could be improved by modeling oxygen solubility as a function of temperature, and it is known that oxygen is less soluble in liquid medium at higher temperatures. This is not likely to impact the model to a great extent because the solubility of oxygen does not vary significantly on the interval of temperatures that the design variable is constrained to, but still presents the opportunity for future modeling.

References

Alibaba. L-lysine for feed additive. Available at: http://www.alibaba.com/showroom/lysine.html. Accessed on 27 April 2015.

Brignole, E. and J. McDowell. 2001. Amino Acid Racemization. Today's Chemist at Work 6(2): 1532-4494. http://pubs.acs.org/subscribe/archive/tcaw/10/i02/html/02brignole.html. Accessed January 25, 2015.

Demirci, A. 2015. BE 468 Lab Manual. Pennsylvania State University, University Park, PA.

Choon Lee, F., G. Pandu Rangaiah, A. Kumar Ray. 2007. Multi-Objective Optimization of an Industrial Penicillin V Bioreactor Train Using Non-Dominated Sorting Genetic Algorithm. Wiley InterScience. DOI 10.1002/bit.21443.

Kerr B.J., Y. Nienaber, and Easter. 2003. Influences of dietary protein level, amino acid supplementation and environmental temperature on performance, body composition, organ weights and total heat production of growing pigs. Journal of Animal Science. http://www.ncbi.nlm.nih.gov/pubmed/12926782#. Accessed February 12, 2015.

Powell, E., and G. Hill. 2008. Optimization of Continuously Stirred Tank Bioreactor Design for Cost Minimization: Effect of Microbial Species and Operating Conditions. International Journal of Chemical Reactor Engineering. Volume 6 (2008). Article A20.

Kookos. I 2004. Optimization of Batch and Fed-Batch Bioreactors using Simulated Annealing. Biotechnology Progress. 2004, 20, 1285-1288.

Yetilmezsoy, K. 2012. Integration of kinetic modeling and desirability function approach for multi-objective optimization of UASB reactor treating poultry manure. Bioresource Technology 118:89 – 101.

Shah, A. H. et al. 2002. Optimization of Culture Conditions for L-Lysine Fermentation by *Corynebacterium glutamicum*. Online Journal of Biological Sciences 2(3): 151-156.

Razak, M. A., B. Viswanath. 2015. Comparative studies for the biotechnological production of L-Lysine by immobilized cells of wild-type Corynebacterium glutamicum ATCC 13032 and mutant MH 20-22 B. Springer. DOI 10.1007/s13205-015-0275-8.

Yao, H.M., Y. C. Tian, M.O Tad, and H.M. Ang. 2001. An Integrated L-Lysine Fermentation Model with Agitation Speed as the Manipulated Variable. Dev. Chem. Eng. Mineral Process., 9(IR). pp, 77-87.

Shuler, M. and F. Kargi. 2002. Bioprocess Engineering- Basic Concepts, Second edition, 263-266. Upper Saddle river, NJ: Prentice Hall PTR.

Heinzle, E., A. Biwer, and C. Cooney. 2006. Development of Sustainable Bioprocess. West Sussex, England.

Y. Toride. 2002. Lysine and other amino acids for feed: production and contribution to protein utilization in animal feeding. Protein Sources for the Animal Feed Industry. Ajinomoto Co., Inc. http://www.fao.org/docrep/007/y5019e/y5019e0a.htm. Accessed January 25, 2015.