L-lysine Production by *Corynebacterium glutamicum*

Clay Swackhamer
Danielle Lesso
Casey Hall
Kashne Kamalanathan

BE 468

Microbiological Engineering

Department of Agricultural and Biological Engineering

The Pennsylvania State University

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Objective

The objective is to produce industrial amounts of L-lysine for use as an animal feed additive. The goal is to produce 25,000 tons, which is 3.125% of world production and 10% of United States production.

Introduction

Lysine is produced for both human consumption and animal feed. It is one of the nine essential amino acids, which cannot be biosynthesized and thus must come from a dietary source (Anastassiadis, 2007). All amino acids contain a chiral carbon, an amine group, and a carboxyl group, as well as an "R" group, which is different for each amino acid. (Lysine K (Lys). 2003). L-Lysine is a basic amino acid, as the amine group tends to bear a positive charge (-NH3+) and its PI is 9.75 (Lysine K (Lys). 2003). The PI is the isoelectric point of an amino acid, 9.75 for L-Lysine.

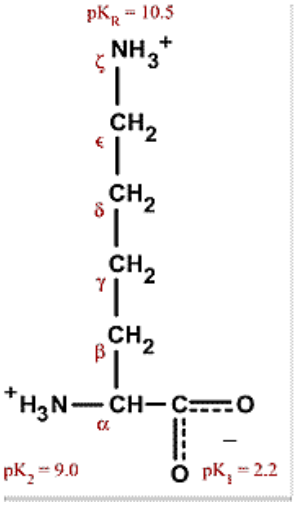

Figure 1. Structure of L-Lysine (Univ. of Maryland, 2003).

In humans L-Lysine has been demonstrated as an important nutritional component, and is especially important for healthy skin, nails, and even hair, as it is necessary for collagen production (Ehrlich, 2011). There is also evidence that L-lysine consumption prevents the Herpes simplex virus from entering its lytic cycle (Ehrlich,

2011), and because of this is used as a preventative or maintenance therapy against outbreaks of this virus. Since there are no biosynthetic pathways for L-Lysine in animals it can become a limiting factor in protein synthesis and thus growth in agricultural production of animals (ADM, 2015). It provides proper digestible amino acid levels, which are critical for production of lean meat. Furthermore, supplementing amino acids in animal feed allows dietary crude protein to be reduced overall, and for every one percent decrease in dietary crude protein nitrogen in manure is decreased 10%, ammonia emissions into the air decreased by 10%, water consumption of the animal decreased by 3% and manure volume decreased by 5% (Kerr et al., 2003).

These may appear to be trivial benefits, but because of the massive scale of the global agricultural industry, even modest improvements in optimizing animal processes are significant. There is an estimated global population of 19 billion chickens kept for agricultural purposes at any given time (Counting Chickens, 2011). With the estimated figure for manure production of chickens, (0.21 lb./day/animal) (Tao et al., 2008) the manure production from chickens can be estimated as 1.89 billion pounds per day, and even a 5 percent decrease in manure production would result in 94.5 million less pounds of manure each day that would need to be treated (Tao et al., 2008). Nitrogen production is estimated as 0.0029 lb Nitrogen/day/animal and using this in addition to the potential for a 10% decrease in animal manure nitrogen content though overall crude protein decrease, a 5.5 million pound per day decrease in the nitrogen produced globally by the poultry industry can be calculated (Tao et al., 2008). These sample calculations show the potential for using L-lysine supplementation to optimize animal diets and also alleviate stress on the environment caused by agricultural byproducts.

The main difference between L-lysine produced for human consumption and that produced for animal feed is the level of purification of the final product. For human consumption, the form is typically that of a fine chemical used as part of a supplement or higher chemical application; in contrast, lysine for animal feed can be within the purity range of 35 to 80% (Anastassiadis, 2007). To determine the purity of the final product, downstream processing options including ion exchange chromatography and drying can be chosen. In this application of lysine production, the objective is to create a lysine product suitable for animal feed.

Use of traditional organic synthesis to create lysine results in 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. In the case of commercial scale L-Lysine production, large investments in capital are required in order to construct and operate the infrastructure through which the fermentation is conducted, which includes reactors, heat exchangers, pumps, storage tanks, and downstream processing equipment (Humphrey and Lee, 1992).

Production Capacity

Overall annual world production of L-lysine is about 800,000 tons (Anastassiadis, 2007). Assuming owning 3.125% of the worldwide market, the objective for annual production of final product was selected to be 25,000 tons. The final product is about 60% pure, calculated on dry matter of L-lysine along with an equivalent amount of hydrochloric acid.

Microorganism

The organism *Corynebacterium glutamicum* is used in industry to produce L-lysine through an aerobic process (Anastassiadis, 2007). Figure 2 shows the biosynthesis of L-Lysine, in which a pathway is followed leading from aspartate (which is itself derived from oxaloacetate, a Krebs cycle intermediate) through a series of

intermediates, all the way to L-Lysine. Pertinent enzymes are shown on the left, and this shows that significant attention can be expended on strain development and metabolic pathway engineering to tune the expression of all of these components and boost production. According to Anastassiadis (2007), "Process improvement for producing larger amounts of L-lysine using microorganisms remains a continual attempt, whereas the continuous development of classical and modern genetics resulted in the development of superior strains imparted with properties advantageous for the commercial production of L-lysine." For this project a culture will be ordered from a cell culture bank, such as the American Type Culture Collection. An example culture that is known to be effective is labeled as ATCC13032 (Käß et al., 2014).

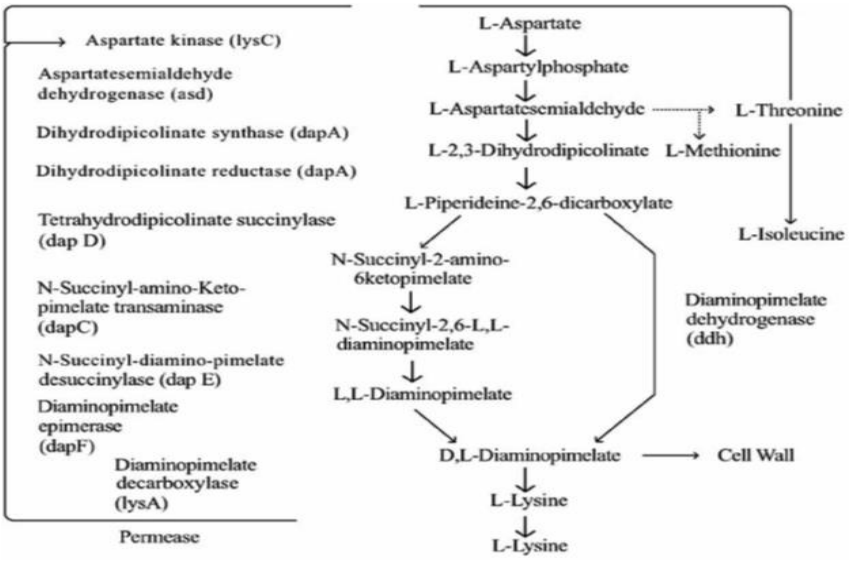

Figure 2. Biosynthesis of L- Lysine (Anastassiadis, 2007)

The organism selected to carry out the production of L-lysine is *Corynebacterium glutamicum*. This microorganism is a gram-positive, non-sporulating, non-motile, ellipsoidal bacteria and is aerobic. The strains of this bacteria typically form pale yellow colonies, however some cream-white strands can also occur (Kjeldsen, 2008). *C.glutamicum* strains have been isolated from soils, soils contaminated with bird feces, and manure (Kjeldsen, 2008). This organism is widely used in the production of L-lysine because it is able to metabolize a variety of carbohydrates, alcohols and organic acids as carbon and energy sources for growth and for amino acid production. It

is also able to metabolize inorganic nitrogen sources such as ammonia. L-lysine is produced in about seven or ten steps from its precursor oxalacetate. *C. glutamicum* has a respiratory metabolism, with oxygen as its terminal electron acceptor. In addition, nitrate can serve as the electron acceptor (Kjeldsen, 2008).

Fermentation Parameters

With the organism selected, more parameters were assigned such as specific growth rate, production rate, and final batch product concentration, and are summarized in table 1.

Table 1: Fermentation Parameters

Parameter	Value	Unit
Production target	25,000	tons
Specific growth rate	0.28	1/hr
Final product concentration	70	g L-lysine/L

These values are necessary when determining size of the reactor, inputs, medium composition, and more. Specific growth rate is strain-specific; other parameters are dependent on fermentation medium and operation conditions.

Fermentation Conditions

When producing industrial amounts of L-lysine, it is important to have a controlled environment for the optimal fermentation process. *C. glutamicum* is found to grow well within a temperature range of 25° to 37° C with an optimum temperature of 30° C, set with a pH range of 7.2 to 7.4 (Ozcan, 2007). In addition, *C. glutamicum* has a high demand for oxygen, therefore it is important to have an efficient oxygen supply present for efficient growth and lysine production (Kjeldsen, 2008). For the culture to grow, it should be aerated at an approximate rate of 0.83 vvm (Guillouet and Engasser, 1995).

Table 2: Growth Parameters for *Corynebacterium glutamicum* (Kjeldsen, 2008)

Growth Parameters	**Value**
Temperature Range	25-37° C
pH Range	7.2-7.4
Aeration Rate	45-52 L air/ L medium/h (0.833 vvm)

Fermentation Medium

C. glutamicum can use a wide variety of carbon and nitrogen sources for production. The growth medium used in the microbial fermentation of *C. glutamicum* is a combination of many different materials from macronutrients to micronutrients comprising mostly of water, sucrose from sugar beets, and corn steep liquor. For the purposes of this study, the sources chosen were the sucrose from sugar beets as the carbon source and corn steep liquor for the nitrogen. Following and adaptation of US patent US5268293, the fermentation medium will consist the components in table 3 below.

Table 3. Fermentation Medium for L-Lysine Production

Ingredient	Amount
Sucrose	75 g/l
ammonium sulfate	40 g/l
CaCO3	40 g/l
Corn Steep Liquor	100 g/l
KH2PO4	1 g/l
MgSO4	0.4 g/l
$FeSO_4\ 7H_2O$	0.01 g/l
$MnSO_4\ 4H_2O$	6 mg/l
biotin	300 µg/l
thiamine-HCl	500 µg/l
pantothenic acid	0.01 g/l

Carbon Source

The chosen carbon source was sugar beets due to their inexpensive cost and availability in the North-central United States. In 2012-2013, the national average cost for sugar beet was $66.60/ton. (USDA, 2014) This ensures that a cheap and renewable carbon source will be available with a composition of up to 22% sucrose (Clark, 2014).

Nitrogen Source

Corn Steep Liquor will be the nitrogen source that is utilized, as it is an economical source of the nitrogen which is necessary for the growth of *C. glutamicum.* This is a reasonably priced nitrogen source that doubles as a finite substance for the growth of *C. glutamicum.* Current prices of Corn Steep Liquor powder range between $500-$1000 per ton, which is comparable in price to other industrial nitrogen sources but because of its composition can also be used as a carbon source, and thus serves dual purpose in this fermentation (Alibaba, 2015). Alternatively,

protein hydrolyzate, corn steep liquor, meat extract or yeast extract containing nitrogen substances can be added (Pythia, 2006).

Micronutrients

Micronutrients are necessary for a variety of reasons. Iron is important for its participation in electron transport chain components, Magnesium as a cofactor for DNA repair proteins, and Biotin (B7) for fatty acid biosynthesis, gluconeogenesis, and amino acid metabolism (Aragony, 2013). Other compounds are used as growth factors, cofactors, or can play a role in ion-exchange. Covalent bonding is used for enzyme immobilization, however the functional groups on cell and support material surfaces are not suitable for covalent binding (Shuler and Kargi, 2002). For this reason, charged ions or ionic compounds such as Fe, Mg, Mn and others are added to fermentation medium to assist in enzyme mobilization.

Some strains of amino acid producing microorganisms are auxotrophic for certain substances, and according to the Pythia Institute of Biotechnology, these mutant strains require the input of these additional substances into the culture medium.

Bioreactor design

Considering the goal yield of 25,000 tons/yr of lysine, several pieces of information must be discovered in order to calculate the number of reactors and volume of reactors for production use. First, the feasible concentration of lysine that will be produced in the fermentation broth is needed, and an acceptable concentration value of 70 g/L was discovered in the literature (Heinzle et al., 2006). Next, equation 1 is devised to calculate the total volume that will need to be processed in order to achieve the yearly production goal, assuming 100 percent product recovery.

$$goal = 50{,}000\ \frac{ton}{year} * \frac{2{,}000lb}{ton} * \frac{0.454\ kg}{lb} * \frac{1000g}{kg} * \frac{1\ L}{70\ g\ L{-}lysine} \qquad \text{Eqn. 1}$$

$$goal = 6.5 * 10^8\ \frac{L}{yr} = 650\ million\ \frac{L}{yr}$$

After the total volume of fermented mediumF is known, the time of fermentation must be calculated. This comes from equation 2, which describes the total fermentation time using its component parts, which are each calculated separately.

$$Total\ fermentation\ time = time_{lag} + time_{log} + time_{stationary} + time_{idle} \quad \text{Eqn. 2}$$

Log phase time is calculated by integrating the first order kinetic equation which describes logarithmic microbial growth, and is shown here in equation 3.

$$\frac{dx}{dt} = \mu x \quad \text{Eqn. 3}$$

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

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

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

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

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 found by analyzing the inoculum concentration and volume of the bioreactor. An assumption must also be made for the relative volume of the inoculum to the reactor, and a standard value of 1% is chosen. For an inoculum grown under the same conditions as the larger scale reactor (20 g biomass / L), the post inoculation biomass concentration in the working reactor (x_o) can be calculated as soon as the volume is known. For now, an assumption for volume must be made, and a figure of 830,000 L is chosen. This is made with knowledge of the general scale of L-lysine

fermentations, and with the knowledge of total required volume per year. It is also calculated in the section on bioreactor design. Using this value, the value of x_o is calculated in equation 4.

$$Conc_{inoculum} * Volume_{inoculum} = Initial\ conc_{reactor} * Volume_{\ Reactor} \qquad \text{Eqn. 4}$$

$$Initial\ concentation_{reactor} = \left(20\frac{g}{L}\right) * (0.01 * 830{,}000L)/830{,}000L$$

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

This is substituted into equation 3 and $time_{log}$ is solved. A value of $\mu = 0.28$ /hr is taken from the literature (Heinzle, et al. 2006).

$$\ln\left(\frac{x}{x_o}\right) * \frac{1}{\mu} = time_{log} \qquad \text{Eqn. 5}$$

$$\ln\left(\frac{20g/L}{0.2g/L}\right) * \frac{1}{0.28/hr} = time_{log}$$

$$time_{log} = 16.4\ hr$$

Next, the other terms in the total time equation are determined. First, idle time is assumed at 10 hr. This includes harvesting, cleaning, refilling, and sterilization. Based on a graph of L-lysine production it can be determined that it is a secondary metabolite, and that the fermentation is type II. This means that significant time must be spent in the stationary phase in order to allow product concentration to rise significantly. From the graph below, the necessary stationary time can be estimated at 35 hr. The lag time can likewise be estimated at 15 hr.

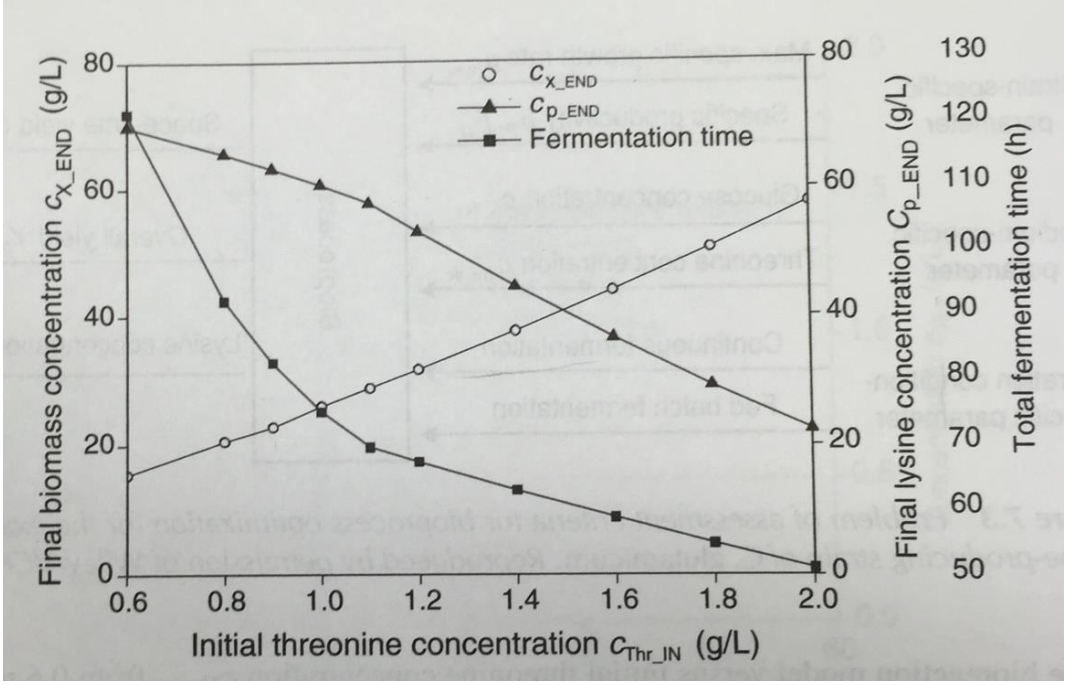

Figure 3. Graph of L-Lysine Fermentation (Shuler, L. Michael. Kargi, Fikret. 2002)

These values are then substituted back into equation 2 for total fermentation time, and this time is evaluated.

$$Total\ fermentation\ time = time_{lag} + time_{log} + time_{stationary} + time_{idle} \qquad \text{Eqn. 6}$$

$$Total\ fermentation\ time = 15\ hr + 16.4\ hr + 35\ hr + 10\ hr$$

$$Total\ fermentation\ time = 81\ hr$$

Next, several calculations are needed to determine the total number of fermentations that can be accomplished per reactor, per year. It is assumed that that production runs for 11 months per year, and this allows the available hours for production to be found, in equation 7.

$$321 days\ x\ \frac{24\ hours}{1\ day} = 7{,}920\ run\ hours \qquad \text{Eqn. 7}$$

$$7{,}920\ run\ hours\ x\ \frac{1\ fermentation}{81\ hours} = 98\ fermentations$$

A value of 96 runs per year is chosen for the reactor, however, in order to allow 2 cycles worth of flexible time (7 days) for maintenance, down time, troubleshooting, and other unforeseen difficulties. It is now evident that a 1.8 million L reactor can be run 96 times per year in our fermentation, but this is not the only way to accomplish the production goal. In fact, there are numerous possibilities to accomplish the goal, by modifying the number of reactors (n) in parallel, and the volume (V) of each reactor. Equation 6 is developed to calculate this production goal.

$$Goal\ \left(\frac{L}{year}\right) = 650 * 10^6\left(\frac{L}{year}\right) = \frac{n\ fermenters}{1} * \frac{Volume\ (L)}{fermenter} * \frac{1}{run} * \frac{96\ runs}{year} \qquad \text{Eqn. 8}$$

The variables in this equation are n and V, and a data set is generated for possible combinations that meet the production goal. Each combination in the following table will allow the production goal to be met, but some are more feasible than others.

Table 4: Combinations of Number Fermenters and Volume per Fermenter that Allow Production Goal to be Satisfied

Number Fermenters (n)	Volume Fermenter (L)
1	3333333
2	1666667
3	1111111
4	833333
5	666667
6	555556
7	476190
8	416667
9	370370
10	333333
11	303030
12	277778
13	256410
14	238095
15	222222
16	208333
17	196078
18	185185
19	175439

The above data set is graphed as a scatter plot, which allows a visual analysis. It can be seen that as the number of reactors is increased, the necessary volume per reactor is lower. Likewise, production could be accomplished with very few reactors if they were extremely large.

A tentative decision has been reached to choose an 830,000 L reactor, and the data set shows that approximately 4 of these reactors will be needed in parallel. This choice may be updated with further optimization when more data is available.

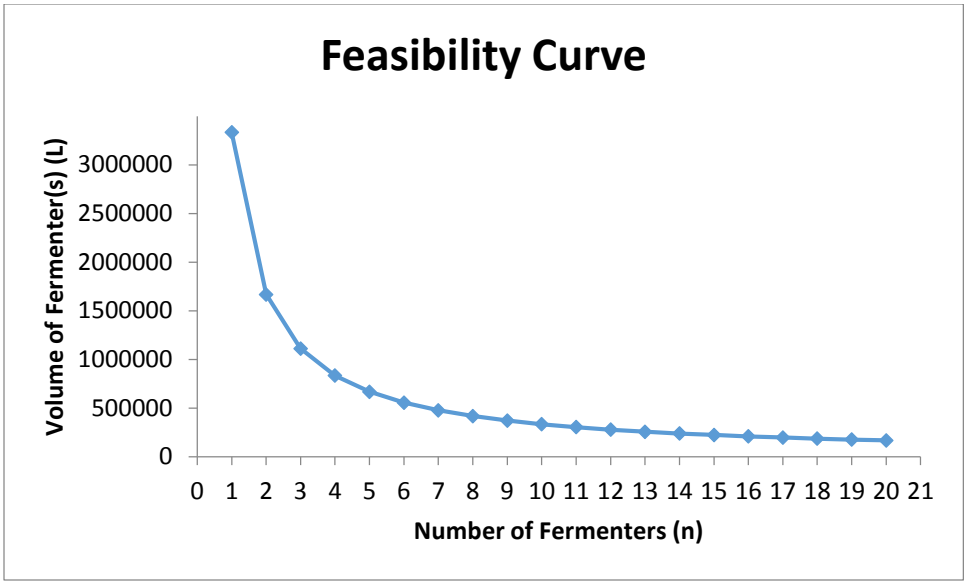

Figure 4. Combinations of Number of Reactors and Volume per Reactor that Satisfy Production Goal

Any point on the line is a combination of points that will accomplish the production goal. Once this information about the working reactor volume is known, the volume of the head space, as well as the dimensions of the reactor, can be calculated by equation 9.

$$Volume\ Head\ Space = 0.2 * Working\ Volume \qquad \text{Eqn. 9}$$

$$Volume\ Head\ Space = 0.2 * 830000\ L$$

$$Volume\ Head\ Space = 1.66 * 10^5 L$$

The total volume is the volume of the head space and the fluid (working volume).

$$Volume_{total} = Volume_{head\ space} + Volume_{working} \qquad \text{Eqn. 10}$$

$$Volume_{total} = 1.66 * 10^5 L + 8.3 * 10^5 L$$

$$Volume_{total} = 9.96 * 10^5 L = 996\ m^3$$

The ratio of diameter to height is estimated based on typical values for large-scale bioreactors.

$$\frac{diameter_{reactor}\,(m)}{height_{\ reactor}\,(m)} = \frac{1}{3} \qquad \text{Eqn. 11}$$

This can be combined with the equation for volume of a cylindrical bioreactor (assuming a flat head plate and base).

$$Volume_{total} = \frac{\pi}{4} * (diameter_{reactor})^2 * height_{reactor} = 996\ m^3 \qquad \text{Eqn. 12}$$

The previous two equations are in two unknowns, and thus the height and diameter of the reactor can be determined.

$$Diameter_{reactor} = 7.5\ m$$

$$Height_{reactor} = 22.5\ m$$

Next, the size of the impeller is calculated, based on a standard equation (13) for this design that is known to lead to adequate oxygen transfer.

$$diameter_{impeller} = diameter_{reactor} * 0.1 \qquad \text{Eqn. 13}$$

$$diameter_{impeller} = 0.75\ m$$

Once the dimensions of the impeller are known, it is important to calculate the length and width of baffles that will be needed to ensure turbulent flow in the tank. These are also based on standard equations, equations 14 and 15.

$$length_{baffle} = 0.5 * \ diameter_{reactor} \qquad \text{Eqn. 14}$$

$$length_{baffle} = 3.75\ m$$

$$width_{baffle} = 0.1 * \ diameter_{reactor} \qquad \text{Eqn. 15}$$

$$width_{baffle} = 0.75\ m$$

These major dimensions of the bioreactor are shown on the following schematic for an easy visualization of the overall system.

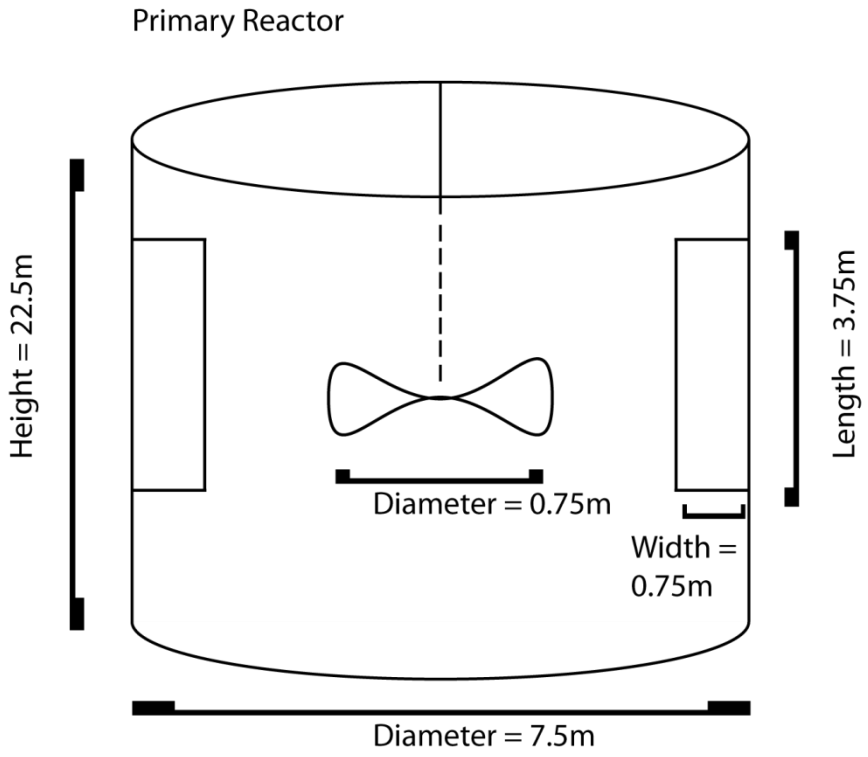

Figure 5. Primary reactor with major dimensions

The calculations for bioreactor design are repeated for the pre-fermenter, which is needed to prepare the inoculum for the primary reactors. With the goal of producing a 1% inoculum for each of the four 830,000L primary reactors running in parallel, a minimum working volume for each pre-fermenter can be calculated. It is assumed that four pre-fermenters will also be used. This will make it easier to coordinate pre-fermentations for the large reactors, which will operate in parallel, but on different schedules.

$$Volume_{working} = volume\ primary\ reactor * percent\ inoculum \quad \text{(Eqn. 16)}$$

$$Volume_{working} = 830{,}000\ L * 0.01$$

$$Volume_{working} = 8{,}300\ L$$

Headspace can be calculated by the same metrics as it was for the primary reactor.

$$Volume_{head\ space} = 0.2 * Volume_{working} \quad \text{Eqn. 17}$$

$$Voume_{head\ space} = 1{,}660\ L$$

Total volume can now be calculated.

$$Voume_{pre-fermenter} = Volume_{working} + Volume_{head\ space}$$

$$Voume_{pre-fermenter} = 9{,}960\ L$$

The ratio of diameter to height is estimated based on typical values for large scale bioreactors.

$$\frac{diameter_{reactor}\ (m)}{height_{\ reactor}\ (m)} = \frac{1}{3} \quad \text{Eqn. 18}$$

This can again be combined with the equation for volume of a cylindrical bioreactor.

$$Volume_{total} = \frac{\pi}{4} * (diameter_{reactor})^2 * height_{reactor} = 9.96\ m^3 \quad \text{Eqn. 19}$$

The previous two equations are in two unknowns, and thus the height and diameter of the pre-fermenter can be solved.

$$Diameter_{pre-fermenter} = 1.61\ m$$

$$Height_{pre-fermenter} = 4.90\ m$$

Next, the size of the impeller is calculated, based on a standard equation (20) for this design that is known to lead to adequate oxygen transfer.

$$diameter_{impeller} = diameter_{pre-fermenter} * 0.1 \quad \text{Eqn. 20}$$

$$diameter_{impeller} = 0.161\ m$$

Once the dimensions of the impeller are known, it is important to calculate the length and width of baffles that will be needed to ensure turbulent flow in the tank. These are also based on standard equations, equations 19 and 20.

$$length_{baffle} = 0.5 * diameter_{pre-fermenter} \quad \text{Eqn. 21}$$

$$length_{baffle} = 0.805\ m$$

$$width_{baffle} = 0.1 * \ diameter_{pre-fermenter} \quad \text{Eqn. 22}$$

$$width_{baffle} = 0.161\ m$$

These major dimensions of the bioreactor are shown on the following schematic for an easy visualization of the overall system.

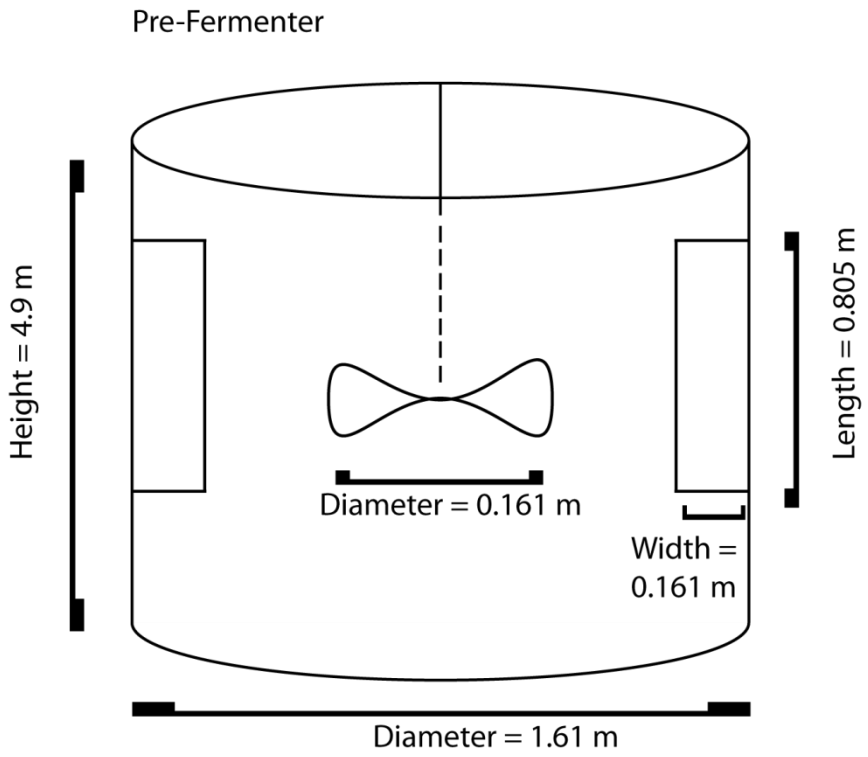

Figure 6. Pre-fermenter major dimensions

Sterilization

Knowing the values for reactor design, the time for sterilization is the last component of reactor design that will be calculated. The primary reactor as well as pre fermenter must be sterilized in order to prevent contamination in the medium from undesired microorganisms. This process is

undertaken at 15 psi and 121°C (Shuler, 2002). The sterilization occurs in-line, as the reactor is pressurized and heated until the desired conditions are reached (Shuler, 2002). These conditions must then be maintained for adequate time in order for successful sterilization, and these necessary times are calculated using known values for the death kinetics of spores as well as the total number of spores in the reactors.

With a probability of unsuccessful sterilization set at the acceptable value of 0.001 (Demirci, 2015), as well as assuming that initial spore concentration is 1*10^5 spores/ L, and that K_d for spores = 1/min (Shuler, 2002), the time for sterilization can be calculated. The last piece of information that is needed is the total number of spores in the primary reactor as well as pre-fermenter, and this information is calculated in equation (21).

Assumptions:

$$\left(1 - P_o(t)\right) = 0.001$$

$$K_d = \frac{1}{min}$$

$$n_{spores} = 1 * 10^5 \frac{spores}{L}$$

Primary Reactor

$$N_{total} = n_0 * V_{working} \qquad \text{Eqn. 23}$$

$$N_{total} = 1 * 10^5 spores/L * 830{,}000L$$

$$N_{total} = 8.3 * 10^{10} \text{spores}$$

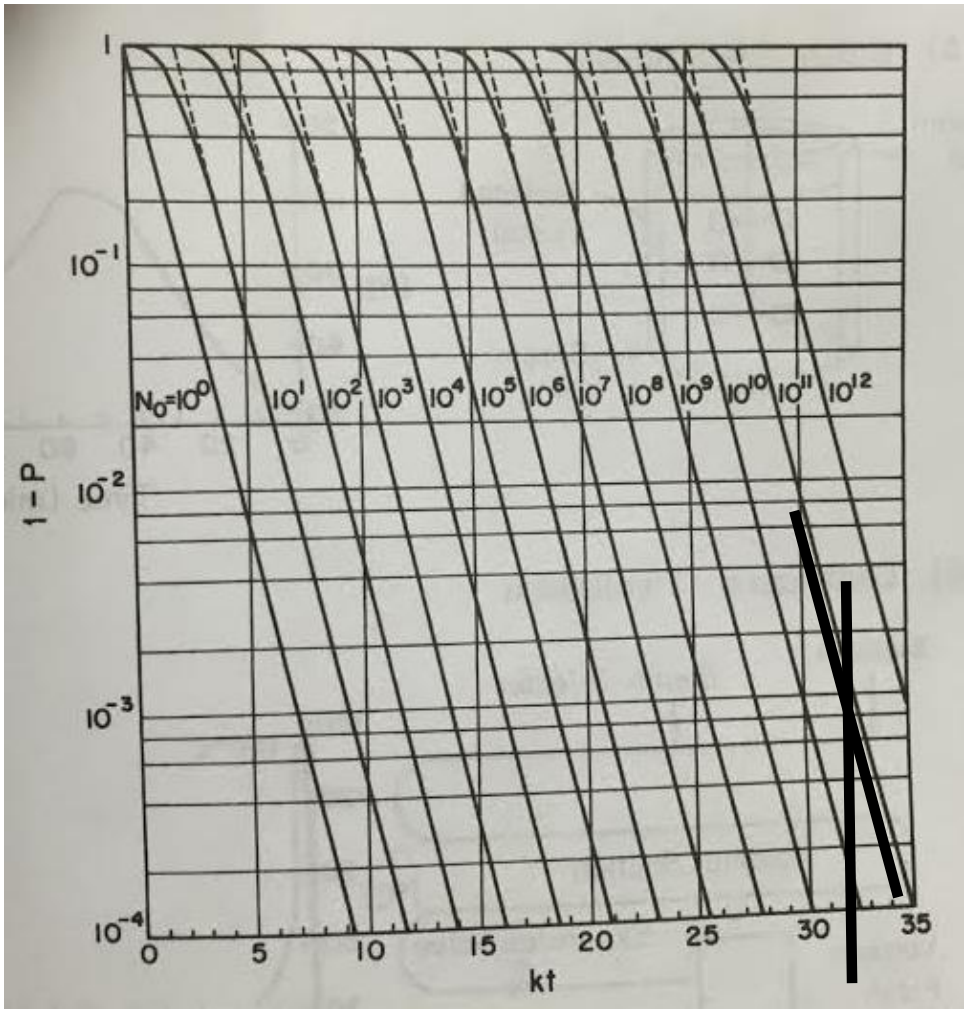

Figure 7. Sterilization chart, conditions marked for primary reactor

$$K_d t \text{ (from figure)} \; 1/32 \; min$$
$$t_{sterilize} = 32min$$

Pre Fermenter

$$N_{total} = n_0 * V_{working} \qquad \text{Eqn. 24}$$

$$N_{total} = 1 * 10^5 spores/L * 9{,}960L$$

$$N_{total} = 9.96 * 10^8 \text{spores}$$

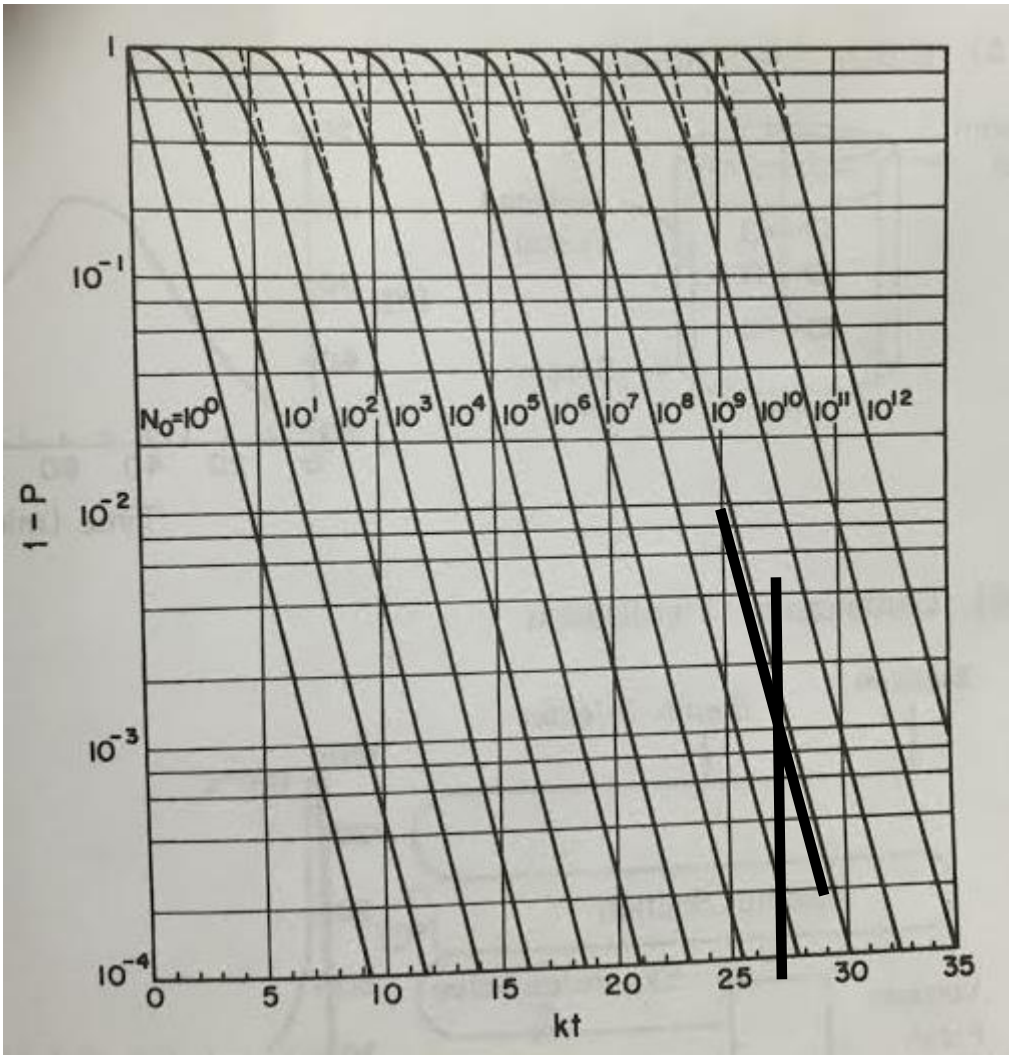

Figure 8. Sterilization chart, conditions marked for pre-fermenter

$$K_d t \text{ (from figure)} = 1/27\ min$$

$$t_{sterilize} = 27\ min$$

Table 5: Values of Design Variables for Pre-fermenter and Main Reactor

Variable	Pre-fermenter	Main Reactor
Working Volume (L)	9,960	830,000
Number Reactors	4	4
Height (m)	4.9	22.5
Diameter (m)	1.61	7.5
Diameter Impeller (m)	0.161	0.75
Width Baffle (m)	0.161	0.75
Length Baffle (m)	0.805	3.75
Time of Sterilization (min)	27	32

Aeration and Agitation:

Aeration

Aeration is the process of introducing gasses into the fermentation broth, and is critically important to the success of industrial fermentations. It is usually accomplished by sparging bubbles of gas into the reactor, and thus must be coupled with pressure exchange systems on the enclosed reactor in order to prevent excessive pressurization (Shuler, 2002). All of these processes must be mediated by sterile filters in order to prevent foreign microorganisms from entering the reactor, but also to prevent the contamination of surrounding air spaces by organisms within the reactor (Demirci, 2015).

Aeration is important because aerobic microorganisms require oxygen. In the case of *C. glutamicum*, an obligate aerobic microbe which has significant demands on oxygen within the

bioreactor, it is evident that some attention must be taken to ensure that an adequate supply of available dissolved oxygen is provided, and this was accomplished through designing an aeration system.

It is necessary to adequately estimate the oxygen requirements for the fermentation of L-lysine, and from a study conducted on a *C. glutamicum* fermentation producing L-lysine, the specific oxygen uptake rate has been determined (Käß et al., 2014).

$$Specific\ O_2\ Uptake\ Rate = 4.0\ \frac{mmol\ O_2}{g_{biomass} * hr} \qquad \text{Eqn. 25}$$

Total oxygen demand depends on the specific oxygen uptake rate as well as active biomass in the reactor, and since biomass changes with time, the instantaneous oxygen demand is dependent on time as well. Biomass concentration as a function of time has been found by integrating the first order kinetic equation for log phase growth of microorganisms and then solving for x. Using this equation, the concentration of active biomass during the log phase can be calculated at any time. It is assumed that before the log phase, the concentration of biomass is equal to the initial concentration (0.2g/L), and that during the stationary phase it is equal to the saturation concentration (20 g/L).

$$\frac{dx}{dt} = \mu x \qquad \text{Eqn. 26}$$

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

$$x = x_o * e^{(\mu * tlog)}$$

Where:

x_o = biomass concentration at time zero, defined as the beginning of the log phase

μ = specific growth rate = 0.28/hr

$tlog$ = elapsed time into the log phase, with the beginning of the log phase defined as $tlog$ = 0

Now that specific oxygen uptake rate and biomass as a function of time are both known, oxygen uptake rate as a function of time can be found, and is presented in equation (13). The advantage of knowing the oxygen demand of the bioreactor as a function of time is that maximum aeration may not be necessary across the entire time of fermentation, and operational costs can be lowered by avoiding unnecessary aeration. For example, early lag phase oxygen uptake may be very low compared to late log phase uptake, and avoiding unnecessary aeration during the times when it is not needed can contribute to cost savings. It is assumed that the concentration of biomass is only changing during the log growth phase. This is because during the lag phase biomass concentration is approximately equal to initial biomass concentration, and during the stationary phase is approximately equal to saturated biomass concentration.

$$Oxygen\ Uptake\ Rate\ (OUR) = Specific\ Oxygen\ Uptake\ Rate * Active\ Biomass\ (t) \quad \text{Eqn. 27}$$

$$OUR = 4.0\ \frac{mmol\ O_2}{g_{biomass} * hr} * \frac{32mg\ O_2}{mmol\ O_2} * x(t)$$

$$OUR = 4.0\ \frac{mmol\ O_2}{g_{biomass} * hr} * \frac{32mg\ O_2}{mmol\ O_2} x_o, for\ \ t < t_{lag}$$

$$OUR = 4.0\ \frac{mmol\ O_2}{g_{biomass} * hr} * \frac{32mg\ O_2}{mmol\ O_2} x_o * e^{(\mu * tlog)}, for\ t_{lag} < t < t_{stationary}$$

$$OUR = 4.0\ \frac{mmol\ O_2}{g_{biomass} * hr} * \frac{32mg\ O_2}{mmol\ O_2} x_{saturation}, for\ \ t > \ t_{stationary}$$

These equations allow OUR to be plotted as a function of time for the entire fermentation.

Figure 9 Oxygen uptake rate as a function of time

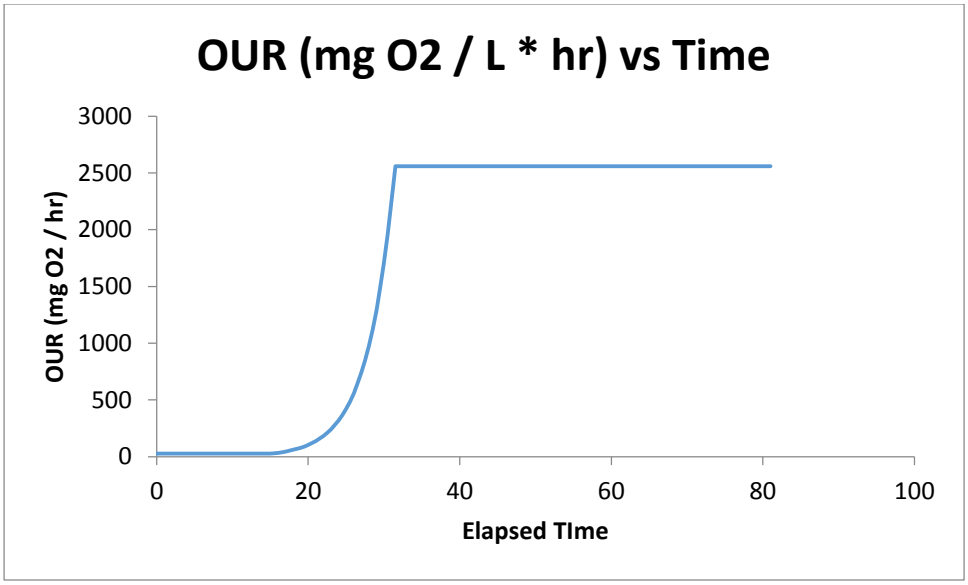

Figure 9. OUR at time t

It is evident that during the lag phase, the microorganisms require modest amounts of oxygen for growth, but that during the log phase this demand rises dramatically, finally plateauing as biomass concentration levels off during the stationary phase at 20 g/L. At this time, the max OUR is reached, which is 2560 mgO2/L. When oxygen transfer is the rate limiting step, oxygen uptake rate equals oxygen transfer rate (Demirci, 2015).

$$OUR\ (demand\ of\ O_2) = OTR(supply\ of\ O_2) \qquad \text{Eqn. 28}$$

Thus, it is necessary to ensure that the demand for oxygen in the bioreactor is met by the supply introduced in aeration and transported from a gas into the dissolved, or liquid, phase. 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, 7.5 mg O_2/L (Demirci, 2015). This value is taken from a standard table.

$$C^*_{L\,std} = 6.8\ \frac{mg\ O_2}{L} \qquad \text{Eqn. 29}$$

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

$$log_{10}(Partial\ pressure\ water\ vapor) = \ 5.4 - \frac{1839}{(T\ in\ °K - 31.7)} \qquad \text{Eqn. 30}$$

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

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

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.

$$P_{abs} = P_{gage} + P_{atm} \qquad \text{Eqn. 31}$$

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

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

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 32 (Demirci, 2015).

$$C^*_{L\,calculated} \ = \ C^*_{L\,std} * \frac{(P_b - P_v)}{(P_{atm} - P_v)} \qquad \text{Eqn. 32}$$

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 $\frac{mg\ O_2}{L}$

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

Next, the necessary transport coefficient K_La can be calculated based on equation (34). The calculated value for C_L^* is used, as is the maximum oxygen uptake rate. This is because the maximum oxygen requirement of the cells must be achievable by the supply. This is important to recognize, because during the log growth phase, performance will be reduced if this transport capacity is not provided, and oxygen uptake rate would exceed oxygen transfer rate. A value of C_L, the concentration of O_2 in the bulk liquid at time t, must also be assumed. In the literature, it is found that aerobic fermentations of *C. glutamicum* are typically carried out at 30% DO, and this means that the concentration of oxygen is equal to 30% of the equilibrium concentration.

$$C_L = 0.3 * C_L^* \qquad \text{Eqn. 33}$$

$$C_L = 0.3 * 9.2\ \frac{mg\ O_2}{L}$$

$$C_L = 2.76\ \frac{mg\ O_2}{L}$$

The necessary value of K_La as a function of time is calculated, and the maximum value reported. The maximum necessary value of K_La is required when the fermenter has maximum oxygen demand (OUR) which occurs at maximum biomass concentration, which is achieved at the end of the log phase and the stationary phase.

$$OTR(t) = OUR\ (t) = K_La * (C_L^* - C_L) \qquad \text{Eqn. 34}$$

$$OTR = 4.0\ \frac{mmol\ O_2}{g_{biomass} * hr} * \frac{32mg\ O_2}{mmol\ O_2} x_o * e^{(\mu * tlog)} = K_L a * (C_L^* - C_L)$$

$$K_L a = \frac{(4.0\ \frac{mmol\ O_2}{g_{biomass} * hr} * \frac{32mg\ O_2}{mmol\ O_2} x_o * e^{(\mu * tlog)})}{(9.2\ \frac{mg\ O_2}{L} - 2.76 \frac{mg\ O_2}{L}\ )}$$

$$K_L a_{max} = \frac{398}{hr}$$

Agitation

Agitation is a necessary feature in bioreactors. In addition to simply homogenizing the fermentation broth, agitation promotes heat transfer and reduces product and biomass gradients that are common in large-scale industrial production (Käß et al., 2014). Another useful benefit occurs when paired with aeration. By using an impeller for agitation, oxygen or air bubbles are of reduced size. Smaller bubbles lead to a larger surface area, which ultimately aids in oxygen transfer from the air inlet to the medium. Breaking the straight path of bubbles from the sparger to the head space will also increase residence time of bubbles in the media, which further improves mass transfer.

Agitation parameters are based off of necessary power requirements and impeller selection. Turbulent mixing conditions can be assumed for proper agitation. As such, a Reynold's number (R_{ei}) of 25000 was chosen.

$$Re_i = \frac{\rho_L * N_i * D_i^2}{\mu_L} = 25000 \qquad \text{Eqn. 35}$$

Properties of the fermentation media will greatly affect the characteristics of flow within the reactor. As opposed to water, media has added components that influence its viscosity. Specifically, media will be more resistant to flow. A viscosity (μ) of 0.05 kg/m-s was found in literature for a typical medium (Jursten, 1998). Using this value, a flat-blade Rushton turbine was selected as the

impeller type. As a radially-directing impeller, it is cost effective and useful for relatively low-viscosity media. To size the impeller, the size of the bioreactor must be considered. Typically, impeller diameter is 10% of that of the reactor.

With radial impellers, vortexes can be created in the media. The solution to this is introducing baffles along the edges of the reactor. Baffle lengths are typically 5% of reactor diameter. The length and width of the baffles as well as the diameter of the impeller are calculated in the bioreactor design section and restated as follows:

$$D_{impeller} = 0.75 \text{ m}$$
$$L_{baffle} = 3.75 \text{ m}$$
$$W_{baffle} = 0.75 \text{ m}$$

Another property of medium that affects impeller behavior is density. By using partial densities of major constituents, a medium density is based off of partial densities. Total density can then be calculated using a weighted average to arrive at a density of 1175.16 kg/m^3.

Table 6: Partial density data for fermentation medium

Ingredient	**Concentration (g/L)**	**Partial density(kg/m^3)**
Water	743.59	1000
Sucrose	75	1590
ammonium sulfate	40	1770
calcium carbonate	40	2710
Corn Steep Liquor	100	1300
KH2PO4	1	2340
MgSO4	0.4	2660
$FeSO_4\,7H_2O$	0.01	1900
Biomass	20	1200

$$density = \sum \frac{concentration\left[\frac{g}{L}\right] * partial\ density\left[\frac{kg}{m^3}\right]}{1000} = 1199.5\ \frac{kg}{m^3} \qquad \text{Eqn. 36}$$

After finding density, all variables are known for calculating angular impeller speed. Other variables are Reynold's number, viscosity, and diameter of impeller (equation 35).

$$N_i = \frac{Re_i \mu}{\rho D_i^2} = \frac{(25000)(0.05\frac{kg}{m-s})}{(1199.5\frac{kg}{m^3})(0.7514\ m)^2} = 1.39\ \frac{1}{s} * \frac{60\ s}{min} = 83\ rpm \qquad \text{Eqn. 37}$$

It is important to consider the amount of power used for impeller input. To do this, the first step is to calculate the power number (P_n). It is determined by using Fig () and parameters Reynold's number and impeller type.

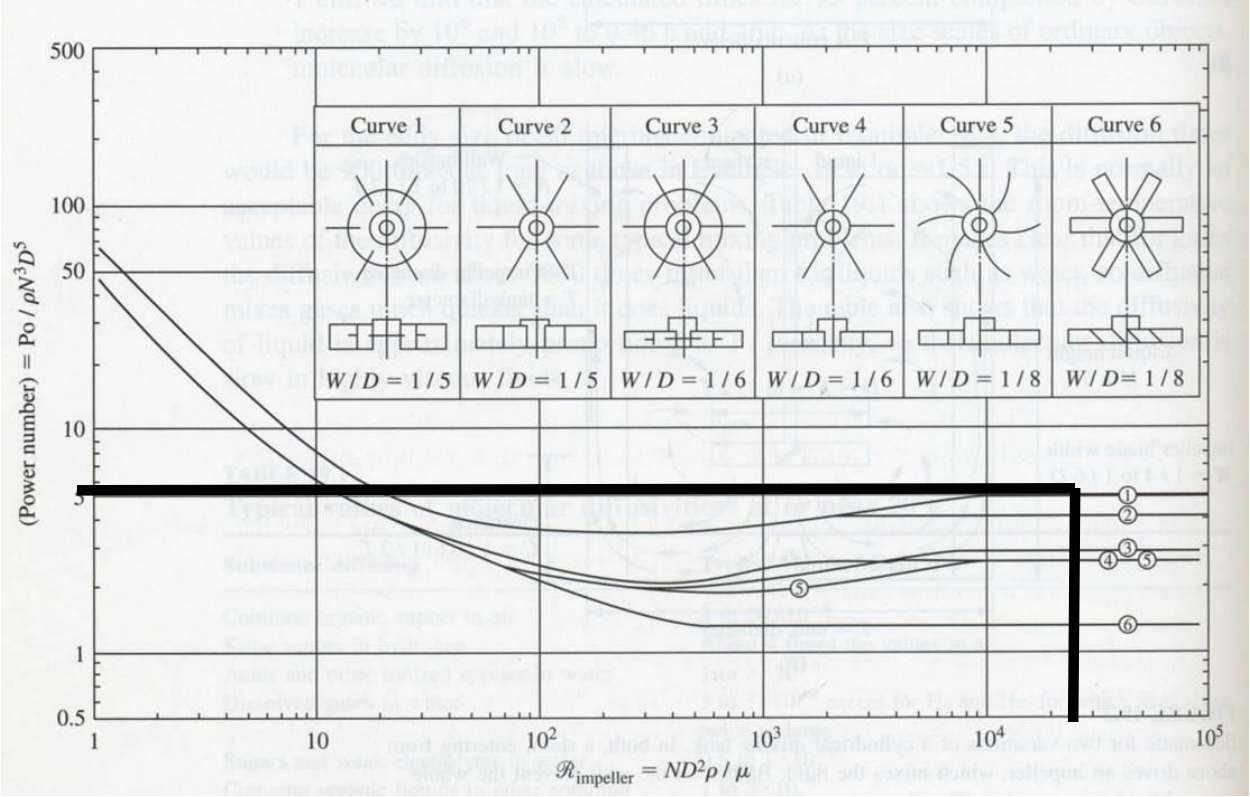

Figure 10. Impeller power number

Considering using a Rushton turbine and assuming six blades, power number P_n is determined to be 5. With power number calculated, it is possible to determine power input (P_g) Eq (368.

$$P_g = P_n * \rho_L * N_i^3 * D_i^5 = 5 * \left(1199.5\ \frac{kg}{m^3}\right) * \left(\left(83\frac{1}{min}\right) * \left(\frac{min}{60\ s}\right)\right)^3 * 0.75m^5 \quad \text{Eqn. 38}$$

$$P_g = 5 * \left(1199.5\ \frac{kg}{m^3}\right) * \frac{2.84}{s^3} * 0.75m^5$$

$$P_g = 3,767\ \left(\frac{kg*m^2}{s^3}\right)$$

$$P_g = 3,767\ \left(\frac{kg*m}{s^2}\right) * \frac{m}{s}$$

$$P_g = 3,767\ N * \frac{m}{s}$$

$$P_g = 3,767\ \frac{J}{s}$$

$$Power = 3{,}767 \text{ Watt}$$

Next, the value of K_La is used to calculate the gas exit velocity, which is the velocity by which gas particles are produced from the sparger.

$$K_L a = 0.5 * \frac{P_g}{Volume}^{0.4} * V_s * N^{0.5} \quad \text{Eqn. 39}$$

$$V_s = \frac{\frac{398}{hr} * \frac{hr}{3600\ s}}{0.5 * \left(\frac{85}{min} * \frac{min}{60s}\right)^{0.5} * \left(\frac{3{,}767\ W}{833\ m^3}\right)^{0.4}}$$

$$V_s = 0.10159\ m/s$$

Where:

V_s = superficial gas velocity (m/s)
N = rotational speed (rev/min)
P_g = Impeller Power input = 3,964 Watt

Volume = bioreactor volume =833 m^3

An assumption for orifice size and geometry must now be made. Orifices are assumed to be circular, with a diameter of 15 cm. The area of a single orifice is calculated with equation 40.

$$Area_{orifice} = \frac{pi}{4} * diamter_{orifice}{}^2 \qquad \text{Eqn. 40}$$

$$Area_{orifice} = 0.01767\ m^2$$

The total number of orifices must be calculated based on gas velocity and air flow rate. The air flow rate in volume air/ volume media/ minute is assumed to be 0.1. This total number of orifices is calculated by equation 41.

$$Air\ flow\ rate = V_s * Atotal \qquad \text{Eqn. 41}$$

$$Air\ flow\ rate = V_s * A_{orifice} * N_{orifices}$$

$$N_{orifices} = \frac{\frac{0.1\ m^3 air}{m^3 media * min}}{0.01767\ m^2 * 0.0995 \frac{m}{s} * \frac{60s}{min}}$$

$$N_{orifices} = 0.928\ \frac{orifices}{m^3 media}$$

The total number of orifices is calculated from the number of orifices per unit volume media, knowing that the working volume of each bioreactor is 833 m^3.

$$Number\ orifices\ total = 0.928\ \frac{orifices}{m^3 media} * 833 m^3 \qquad \text{Eqn. 42}$$

$$Number\ orifices\ total = 774$$

In conclusion, and aeration and agitation system was designed for the bioreactor, which will produce L-lysine. Several assumptions have been made, such as air flow rate and orifice size, based on reasonable knowledge of these parameters in other systems. The assumption has been made that

the fermentation will be oxygen limited, that is, that while the system is operated the oxygen uptake of the microorganisms will equal the oxygen transfer provided by aeration and agitation. The overall system is now ready for further design considerations, such as product recovery and economic analysis.

Downstream Processing

At this stage of the analysis, it is important to recognize the steps of production that have already been elaborated, and determine the remaining steps as well. The formulation of medium, fermentation parameters, and bioreactor design have all been accomplished, as has the design of a sufficient aeration system and agitation system. The growth of the microorganism has been quantified and production of product calculated. However, these steps are all part of primary fermentation, and the result of these processes is still a dilute and multi-component solution of dissolved product, which is of relatively little value without further processing. What is needed is an effective way to both purify and concentrate the product into a form that can be sold. While designing this process, several general heuristics for bioprocessing are kept in mind.

First, major impurities such as spent fermentation medium and biomass are to be removed as early as possible in the overall scheme of processing (Zydney, 2015). This is because the earliest possible removal of impurities allows the feed stream to be scaled down in volume, and thus subsequent processing equipment can be designed at a smaller scale, which leads to reduced costs. Second, the product is to be moved through the overall process as quickly as possible (Zydney, 2015). This prevents product breakdown as well as frees up equipment to process more batches in the same amount of time, lowering labor and energy costs. Third, it is desired to remove general impurities early, and then remove specific impurities with targeted purifications (if necessary) once

the feed stream has been scaled down, concentrated, and general impurities removed (Zydney, 2015). This is because general impurities usually can be removed without impacting yield, whereas obstinate specific impurities can sometimes only be reduced to acceptable levels using processes which impact yield more severely, and it is desired to eliminate any steps early on in the process which significantly decrease yield. In addition, specific impurity removal usually functions better at smaller scales of volume and with highly concentrated product, and this is more easily achievable if these steps are accomplished later in the processing. Lastly, specific impurity removal often involves specialized and expensive equipment, and these costs can be reduced once the feed stream has been concentrated (Harrison, 2003).

In the overall scheme of processing, it is necessary to determine whether the processing steps will occur in batch or continuous form. In the case of L-lysine production, where the primary fermentations are all batch processes, it is desired to move forward with batch processing as well. This allows for better coordination between primary fermentation and downstream processing. It is acknowledged that continuous processing is theoretically possible, but at this moment it is unlikely that this is performed by any production group on an industrial scale (Wendisch, 2007).

It is also necessary to determine in what form the product is present in the fermentation medium. In the case of L-lysine, which is secreted as an extracellular product, it is in solution (Wendisch, 2007). This means that a good candidate for an initial step is the removal of biomass. This is accomplished via rotary vacuum drum filtration.

In this step, the bioreactor is emptied slowly into a large tank, in which a partially submerged drum covered in filtration medium is housed. This drum rotates and a vacuum is drawn in the center, pulling broth out of the tank while filtering biomass. The biomass cake is continuously scraped off

with a fixed knife and disposed, while the clarified broth is advanced to the next step in purification (Harrison, 2003).

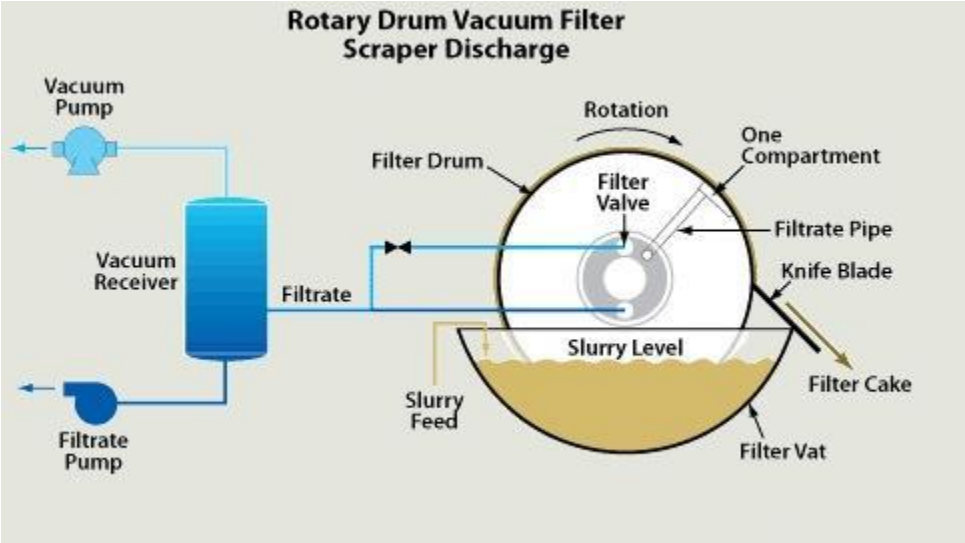

Figure 11. Rotary drum vacuum filter schematic (Osborne and Pierson, 1990)

A total filtration time of 5 hours was targeted for the entire rotary drum, which includes the actual filtration process in the submerged slurry along with the formation of the cake around the drum separating it from the clarified broth. Therefore, to filter through 830m^3 of multi-component solution of dissolved particles, it was assumed that about 20% of the drum was submerged at a speed of 0.167 revolutions per second, which is equal to 10 revolutions per minute. From the assumed speed of the drum rotating along with the percent submerged it was made possible to calculate the time of the filtration process in order to use a manipulated version of the Ruth equation to solve for area required for the drum. Several parameters to be considered for calculating the necessary filter area are presented in Table 7 along with the calculations for area.

Table 7. Filter parameters

Variable	Value	Units
V	830	m^3
α	4	m/kg
C	20	kg/m^3
μ	$1.13x10^{-2}$	kg/m-s
Δ P	0.05	N/m^2
g_c	9.81	m/s^2
r_m	5760	m^{-1}
t	1800	s

The Ruth equation is used to determine filter area needed to process the feed in an arbitrary amount of time, set to seven hours in this case.

Ruth Equation:

$$V^2 + 2VV_0 = kt \qquad \text{Eqn. 43}$$

The equation is linearized, and modified for application to a rotary vacuum filter.

$$\left(\frac{V'}{n}\right)^2 + \frac{2V'}{n}V_0 = kt$$

Where:

Filtrate flux = $V' = \frac{V}{t}$

Volume constant = $V_0 = \frac{r_m A}{\alpha C}$

Proportionality constant = $k = \frac{2A^2}{\alpha C \mu}\Delta P g_c$

Time constant = $t = \frac{\varphi}{n}$

Rotational speed = $n = 0.167\, rps$

Submerged fraction of drum = φ = 0.2

$$\left(\frac{\frac{830m^3}{1.25s}}{0.167rps}\right)^2 + \left(\frac{2\frac{830m^3}{1.25s}}{0.167rps}\right)\left(\frac{5760m^{-1}A}{4\frac{m}{kg} * 20\frac{kg}{m^3}}\right) = \frac{2A^2}{4\frac{m}{kg} * 20\frac{kg}{m^3} * 1.13x10^{-2}\frac{kg}{ms}}\left(34473\frac{N}{m}\right)1.25s$$

$$A = 18.98\ m^2$$

From rearranging the Ruth equation, the area of the rotary drum vacuum filter was calculated to be 18.98m^2. The dimensions of the filter based off of this area have a radius of 2.45m and an arc length of 1.28m at an angle of 30^o. This is based off of a total rotary drum vacuum cake separation of 5 hours, with a 1.25s actual filtration time in the submerged section of the drum.

The next step in purification is evaporation, in which heat and airflow are used to remove the majority of fermentation medium. This step involves significant energy expenditures, and cannot be tolerated by heat sensitive products, but in the case of L-lysine it is well tolerated and considered industry standard. There are several options for batch evaporation, including rotary vacuum shelf dryers and rotary dryers. A vacuum is involved in evaporation in order to reduce the pressure below the vapor pressure of the liquid, and thus allow evaporation to occur at lower temperature (Harrison, 2003).

After the bulk liquid has been removed by evaporation, the product is obtained in solid form by spray drying. This process uses equipment to atomize the feed and spray tiny droplets into a stream of hot air, which evaporates the liquid and results in tiny dried particles of product. This process allows a dried L-lysine powder to be efficiently obtained, and is described in the schematic Figure 12.

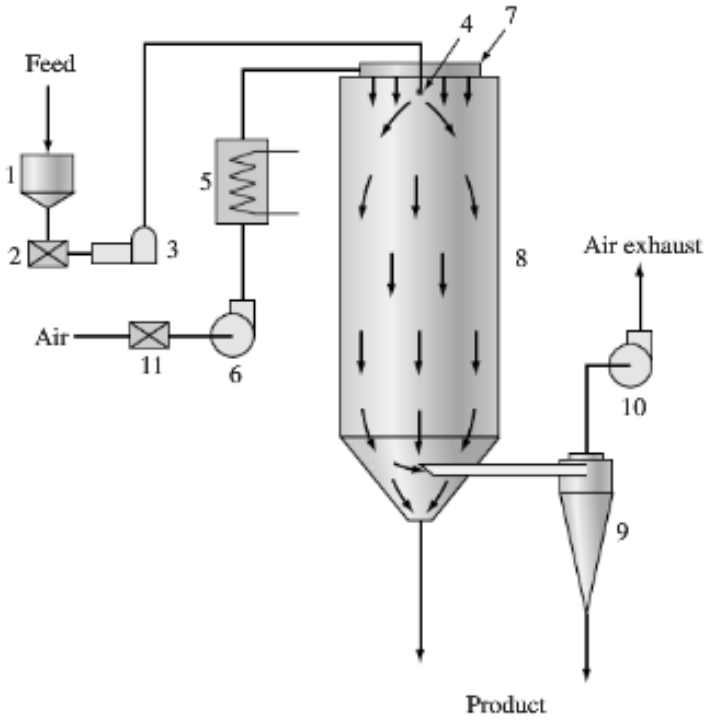

Figure 12. Spray dryer schematic (Harrison, 2003)

Where:

1. Feed tank
2. Filter
3. Pump
4. Atomizer
5. Air heater
6. Fan
7. Air disperser
8. Drying chamber
9. Cyclone
10. Exhaust fan
11. Filter

A limitation of spray drying is that it does not provide any purification. Anything in the liquid medium becomes trapped in the solid particles, which will contain L-lysine, but also a variety of impurities that were present in the medium. For animal feed this is considered acceptable, but for

research or pharmaceutical use a much higher degree of purity would be required. In order to provide the possibility of producing a higher grade of L-lysine, a different recovery process could be used in place of spray drying. A good candidate unit operation for this process is ion exchange chromatography (IEX).

Ion exchange chromatography involves pushing the feed stream through a column that is packed with resin. The product is bound to the resin, then the column is washed with a buffer to expel unbound impurities. Finally, the column is eluted under different conditions than those under which binding occurred (Zydney, 2015). This allows the product to leave the solid phase and re-enter the liquid phase, then become expelled from the column where it can be captured and moved to a higher purity storage. Since L-lysine is positively charged at neutral pH, the column would be packed with an anionic resin. This would allow the binding of the product at neutral pH. Elution would occur at a pH higher than the isoelectic point for L-Lysine, which is 9.75. At this pH, the product becomes neutral and leaves the solid phase. It can then be eluted from the column at both high concentration and purity.

This process is not feasible for the general production of animal feed for several reasons. First, IEX resins and control systems tend to be very expensive, and secondly, function at relatively low flow rates, well below those which would be necessary to process the amounts of L-lysine necessary to have an impact in the market for animal feed (Harrison, 2003). It is nevertheless an interesting addition to the overall process that could function in parallel with spray drying and be used to produce a product at higher purity, which could allow the operation to follow the demands of the market any given time and operate with flexibility that could lead to higher profits.

The overall processing of L-lysine is accomplished using a minimum number of steps, and with overall high yield. It is important to note, however, that this processing is highly specific to production for animal feed, as research or pharmaceutical grade L-lysine will be purified by different steps in order to produce product of a purity not necessary for animal feed grade. The overall schematic of production is shown in Figure 12.

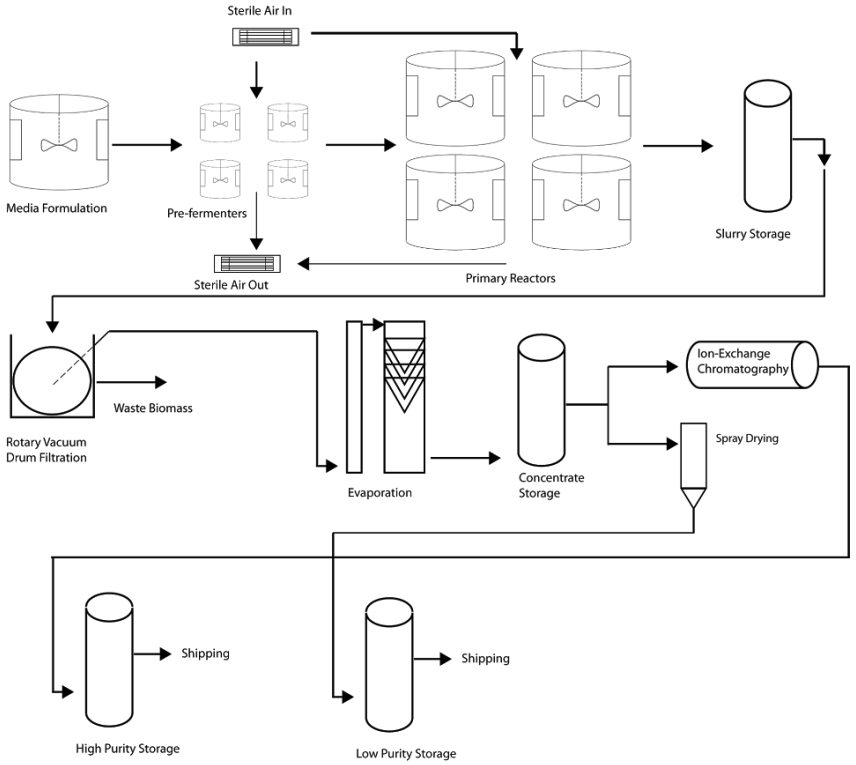

Figure 13. Overall process flow diagram of L-lysine production

Conclusion

As an essential amino acid, lysine is a valuable additive for both humans and animals. Of particular importance is its use as a supplement for animal diets because of its large impact on the agriculture industry. Since it is essential, it acts as a limiting factor in protein synthesis. By increasing lysine intake by farm animals, animal quality can be improved by increasing growth size, increasing

lean meat percentage, and decreasing manure nitrogen levels that lead to ammonia emissions. The global agriculture industry is of a huge scale, and these benefits lead to a more efficient system. As such, this report offered a design for L-lysine production for use as animal feed supplements. This product can be created by a biological step to account for the L-stereoisomer and only needs to be purified to 60%. A goal yield of 25,000 tons/yr was decided.

The lysine was produced by *Corynebacterium glutamicum* through aerobic fermentation. *C. glutamicum* has been widely used to produce L-lysine because it is able to metabolize inorganic nitrogen sources and a variety of carbon sources, making it a very useful microbe for large-scale production. It has an optimal temperature of 30° C and needs an efficient oxygen supply, making aeration and agitation extremely important. With an acceptable concentration of 70 g/L of lysine in the bioreactor, 20 g/L of biomass is needed. The bioreactor has a working volume of 830,000 L and can be run 96 times/ yr. The prefermentor then has a working volume of 8,300 L. Main fermenter is 22.5 m high and 7.5 m wide and supplemented with a 0.75 m impeller running at 18 rpm. With a specific oxygen uptake rate of 4.0 mmol O_2/g biomass-hr, k_La would have to be 0.0034 /s.

After the bacteria produced the expected yield of lysine, downstream processing begins. Specifically, this refers to separation and purification of the broth into the final product. After first filtering the broth by a rotary drum vacuum filter to remove the biomass and other solids, the resulting liquid is evaporated. This final solution is sent through a spray dryer to create isolated particles of product. Using this multistage system, the fermentation broth is transformed into a marketable, useful lysine supplement.

Limitations and Recommendations:

Several limitations of this design process must be recognized. The first assumption made is the production goal (10% of the global market share), which is assumed to be a share which could lead to a profitable operation. Secondly, it is assumed that large scale fermentation will remain to be the most economical option for production of L-lysine. Significant advances in synthetic organic chemistry or chiral molecule separation could theoretically change this. L-lysine is an inherently valuable product because it is an amino acid which is not biosynthesized in mammals. It is thus also assumed that the metabolism of agricultural animals will not be altered through genetic engineering to allow synthesis of L-lysine. This type of major metabolic engineering is considered highly unlikely, and coupled with the growing agricultural industry, L-lysine should remain a useful product for the foreseeable future.

Several fermentation parameters were gathered from literature, but must be considered assumptions that would require experimental validation. These include specific growth rate and the achievable final concentration of L-lysine. Likewise, the aeration rate, optimal temperature, optimal pH, inoculum concentration, saturation biomass, and medium composition are taken from literature detailing optimization efforts for this fermentation, but should also be validated experimentally.

In the calculation of volume needed to cover the production goal, it is assumed that 100 percent of product produced will be recovered and sold. This assumption will need to be adjusted for each individual operation. Idle time, lag phase growth time and stationary phase times were all taken from literature, but should be considered cautiously. It was assumed that production can only run for 11 months of the year, and that the plant can operate on a 24 hour schedule.

It is assume that the head space of the reactor is 20% of the working volume, that ratio of diameter to height is one to three, that the reactor is a perfect cylinder with flat head and base plates, and that it operates at 5 psi (largely determined by the air-out filter). It is assumed that the diameter of the impeller is 10% of the diameter of the reactor, that the length of the baffles is 50% of the length of diameter of the reactor, and that the width of the baffles is 10% of the diameter of the reactor.

It is assumed that an acceptable probability of unsuccessful sterilization is 0.001, that the Kd value for spores is 1/min, and that the concentration of spores in the medium is $1*10^5$/L.

A value for the specific oxygen uptake of the microorganism is taken from literature, and that this value is constant for all times during the fermentation. It is assumed that oxygen uptake will equal oxygen transfer rate during the process, as well as that the fermentation will be carried out for conditions where the concentration of dissolved oxygen is 30% of the maximum concentration.

It is assumed that the Reynolds's number of the medium will be 25,000, as well as that the microorganism can tolerate the shear induced by this agitation. The viscosity of the medium was found in literature but should also be validated. It is assumed that a Rushton type impeller will be used in the reactor. It is assumed that orifices for aeration will be 10 cm in diameter and circular.

For processing, it is assumed that sufficient mass transfer could be achieved in an ion exchange chromatography column without requiring pressure drop greater than the specified allowable drop for an IEX column for the fermentation medium that is used.

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