EVALUATION OF OXYGEN MASS TRANSFER IN FUNGAL FERMENTATION USING AIRLIFT/BUBBLE COLUMN BIOREACTORS

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ABSTRACT

Submerged fungal fermentation using bubble column and airlift bioreactors was investigated for converting bioethanol liquid waste, vinasse into high-value fungal protein for fish or animal feed applications. Previous studies suggested that fungal biomass yields are dependent on many environmental parameters such as: pH, nutrients, temperature, aeration rate, and colony morphology among others. There is a lack of studies that examine the oxygen mass transfer in fungal fermentation. This research closely examined the air-to-liquid oxygen mass transfer coefficient, $k_L a$; an important factor affecting fungal biomass yield. Laboratory-scale, 2.5 L working volume bubble column and airlift bioreactors with sugarcane ethanol process derived vinasse as a substrate and the fungal species *Rhizopus microsporus* var. *oligosporus* were used for oxygen mass transfer studies. Results showed that $k_L a$ followed a power curve for both airlift and bubble column configurations using water-only media for air flow rates ranging from 0.5 to 2.0 volume of air/volume of liquid/minute (vvm). Power regression equations $k_L a_{ALR} = 37.9 * vvm^{0.949}$ and $k_L a_{BLR} = 39.0 * vvm^{0.941}$ correspond to airlift and bubble column configurations, respectively.We determined that $k_L a$ for vinasse media alone (i.e., without fungus), increased for increasing aeration rates as expected for the bubble column configuration from 10.1 ±0.2 h^{-1} to 59.4 ±1.9 h^{-1}, respectively at aeration rates of 0.5 to 3.0 vvm. The $k_L a$ for vinasse media was on average 34.1±17.7 % lower than water at each aeration rate when operated without fungus. Results showed that the presence of fungi in vinasse media resulted in decreases and increases of $k_L a$ compared to vinasse alone at 1.5 vvm. A maximum biological enhancement of +30.7% at 6.53 g was observed. Knowledge of the oxygen mass transfer properties in complex media fermentation is crucial for process scale-up and commercialization of the fungal technology.

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NOMENCLATURE

a, a_s	Volumetric surface area (m^2)
C^*	Saturated dissolved oxygen concentration (%)
C	Dissolved oxygen concentration (%)
C_0^*	Steady state dissolved oxygen concentration in the presence of oxygen uptake (%)
$C_{X,m}, C_{X,L}$	Cell concentration in monolayer and liquid films (cells/mL)
$\frac{dC}{dt}$	Change in dissolved oxygen per unit time (mg/(L·s))
d_s	Bubble diameter (m)
D	Oxygen gas diffusivity (cm^2/s)
E	Biological enhancement factor (dimensionless)
$F_{O_2}^{in}, F_{O_2}^{out}$	Oxygen concentration in inlet and outlet streams (mg/L)
k_L	Oxygen mass transfer coefficient (m^2/h)
$k_L a$	Volumetric oxygen mass transfer coefficient (h^{-1})
$K_L a$	Volumetric oxygen mass transfer coefficient in the presence of respiring organism (h^{-1})
N	Stirred speed (rpm)
Q	Aeration rate (L/min)
q_X	Specific oxygen consumption rate (mg O_2/g biomass)
u_L	Superficial liquid velocity (m/s)
U_s	Terminal rise velocity of the bubble (m/s)
v_g	Air bubble superficial velocity (m/s)
v_{slip}	Bubble slip velocity (m/s)
v_{tip}	Impeller tip speed (m/s)
v_s	Superficial gas velocity (m/s)
X	Cell concentration (g)
V	Bioreactor volume (L)

vvm	Volume of gas/volume of liquid/minute (L/L/min)
$V_{LC}, \bar{V}_{LR}$	Average velocity in the core region and average linear velocity (m/s)
z_m, z_L	Cell and liquid monolayer thickness (μm)

Greek Symbols

ε	Gas hold-up (dimensionless)
μ	Viscosity (kg/(s·m))
τ_{RT}	Dissolved oxygen probe response time (s)
τ_{tip}	Shear stress at the impeller tip (pascal)
ν	Kinematic viscosity(m^2/s)
θ	Mixing time (s)

Acronyms

SSF	Solid-state fermentation
SmF	Submerged fermentation
ALR	Airlift bioreactor
BLR	Bubble column bioreactor
STR	Stirred-tank bioreactor
OTR	Oxygen transfer rate (mg/(L·s))
OUR	Oxygen uptake rate (mg/(L·s))
DO	Dissolved oxygen (%)

CHAPTER 1 INTRODUCTION

Ethanol production by yeast fermentation does not fully utilize the potential energy of the starting raw material. The protein, lipids, and carbohydrates of biomass feedstocks such as corn, sugarcane, sweet sorghum, and sugarbeet are complex and not 100% utilized by yeast fermentation alone. Ethanol production's solid and liquid waste streams are rich in biological potential energy--the liquid waste stream is especially rich in nutrients because of yeast cells left over from fermentation. Developing co-products help to utilize the "waste" streams. Thus, co-products help to decrease the net cost and increase the energy efficiency of ethanol production by finding a source of revenue and purpose for the waste stream. For example, the United State's 2012 corn-ethanol industry produced 13.3 billion gallons of ethanol while co-producing 36.7 metric tons of high-quality livestock feed from 4.8 billion bushels of corn. (Renewable Fuels Association, 2013)

Other researchers have investigated generating co-products from sugar-based ethanol waste streams. Two examples of co-products are, yeast protein concentrate (YPC) for carp feed from bioethanol waste and fungal biomass for protein feed ingredient from bioethanol waste, etc. (Nitayavardhana and Khanal, 2010; Omar et al., 2011)

The focus of this research is based on the study from Nitayavardhana and Khanal (2010), in which they proposed an "innovative biorefinery concept" to increase the value of vinasse. They proposed adding a processing step which involves fungal fermentation using *Rhizopus microsporus* (var. *oligosporus*) on vinasse. Nitayavardhana and Khanal (2010) conducted optimization studies using a series of 250 mL shaker flasks containing 100 mL of media. However, industry processing

volumes for vinasse are on the order of $1x10^{10}$ L/year. Thus there exists a need for industrial scale fermentation strategies.

Sugarcane ethanol is distilled from the broth of yeast fermentation on sugarcane syrup. Distillation leaves a waste, an aqueous solution, known as *vinasse*, which consists of spent sugar-syrup, yeast cells, minerals and salts. Nitayavardhana (2012) reported that vinasse from sugarcane juice has the following characteristics: chemical oxygen demand of 30.4 g/L, biochemical oxygen demand of 16.7 g/L, total nitrogen of 628 mg/L, total phosphorous of 130 mg/L, sulfate of 1.4 g/L, and potassium of 2.0 g/L.

Vinasse has hazardous effects when disposed of improperly to the environment. Traditionally vinasse is disposed by fertirrigation to the agricultural lands. This practice, however, has serious environmental concerns including increasing biological oxygen demand (BOD), causing nitrogen pollution and decreasing the alkalinity of soil. High levels of nitrogen in the water leads to algal blooms and lowered dissolved oxygen (DO) in streams, rivers, and gulfs. Decreased alkalinity of land leads to lower crop yield (Martinelli and Filoso, 2008).

In 2013, Nitayavardhana et al. investigated fungal fermentation using an airlift (ALR) and bubble column bioreactor (BLR) configurations. The use of an ALR and BLR was more ideal because there is no mechanical mixing compared to the traditional stirred tank reactor. A lower shear rate is essential to maintaining the desired morphology of the fungus in pellet form (versus mycelia hair-like structure). Other benefits of the ALRs and BLRs are lower operational cost for bulk liquid mixing (versus mechanical impeller agitation) and lower maintenance costs due to lack of moving parts within the reactor (Smart and Fowler, 1984).

The aerobic nature of *Rhizopus microsporus* requires supply of sufficient oxygen to the media to ensure non-growth limiting conditions in the reactor. Nitayavardhana et al. (2013) 2.5 L working volume ALR and BLR experiments showed that an aeration rate of 1.5 vvm (volumes of air per volume of liquid per minute) resulted in the highest fungal yield: 8.04 ± 0.80 (g biomass increase/g initial biomass). At lower aeration rates (0.5 and 1.0 vvm) dissolved oxygen (DO) concentrations were reduced to near 0 mg O_2/L after 24 hours of fermentation. This coincidence of lower yields at lower aeration rates suggests that insufficient DO reduces biomass yield.

The optimal DO for fungal fermentations is 20-50% of air saturation or about 1.6-4.0 mg O_2/L (Su and He, 1997; Fadenza et al. 2010). DO concentrations can drop substantially during fungal growth with oxygen uptake rate increasing directly with exponential growth phase and increased biomass concentration. Sufficient oxygen mass transfer from gas to liquid (i.e. from air bubble to vinasse) is essential to sustain fungal growth and maximize biomass yield (Rajagopalan and Modak, 1993; Garcia-Ochoa et al., 2010).

One important parameter that describes the oxygen mass transfer in bioreactors is the volumetric oxygen mass transfer coefficient, $k_L a$. $k_L a$ is a coefficient which is determined by the physical and operational properties of the bioreactor. When $k_L a$ is known, the oxygen mass transfer rate (OTR) can be known. However, no studies have measured $k_L a$ for fungal fermentation on vinasse.

Chemical and biological engineers have researched the many aspects and impacts of $k_L a$ in airlift and bubble column bioreactors. The lumped volumetric oxygen mass transfer coefficient ($k_L a$) and it's dependent parameters: liquid

viscosity, gas bubble diameter, gas hold-up, gas-vector-liquid transfer, antifoaming agents, oxygen transfer efficiency, concentration gradients, exposure time, reactor geometry, and biomass oxygen uptake rate, are all subject areas well documented. (Garcia-Ochoa et al., 2000; Garcia-Ochoa and Castro, 2001; Christi and Jauregui-Haza, 2002; Galaction et al., 2004; Kilonzo and Margaritis, 2004; Junker, 2007; Pilarek and Szewczyk, 2008; Garcia-Ochoa and Gomez, 2009; Suresh et al, 2009; Quijano et al., 2010; Garcia-Ochoa et al., 2010) However, how these topics pertain specifically to fungal fermentation in vinasse is yet to be determined. The importance of $k_L a$ and $k_L a$ with fungus ($K_L a$) on bioreactor design and its effects on biomass yield warrants the investigation of $k_L a$ and $K_L a$ in fungal fermentations on vinasse substrate in airlift and bubble column bioreactors.

By understanding the oxygen mass transfer properties of ALRs and BLRs in fungal fermentations, engineers can reduce aeration costs by only supplying the minimum amount of oxygen required to produce the maximal amount of fungus. Reducing the operating costs of the co-product production is a crucial step to making it economical for use in the ethanol industry. Furthermore, by including a fungal fermentation step in the ethanol process, the environmental impact of vinasse on the water quality will be reduced

The objectives of this study was to investigate the oxygen mass transfer of fungal fermentation using *Rhizopus microsporus* (var. *oligosporus*) on vinasse in 2.5 L laboratory scale airlift and bubble column bioreactors. First by measuring $k_L a$ under water-only and vinasse-only conditions at various aeration rates in ALR and BLR configurations, $k_L a$ values can be estimated for the abiotic media. The second objective was to measure the volumetric oxygen mass transfer coefficient during

fungal fermentation $K_L a$. $K_L a$ is denoted with a capital K to signify there is a fungus present (i.e. there is oxygen consumption) in the media.

CHAPTER 2 LITERATURE REVIEW

2.1 Fermentation Technology

2.1.1 Submerged Fermentation

Erlenmeyer Flasks

The Erlenmeyer flask is a small, laboratory scale cultivation vessel (0.25-1.00L) that can be used for fungal growth on vinasse. The Erlenmeyer flask can maintain a closed environment at a low cost which makes it ideal for primary experiments. Within an incubator shaker, an Erlenmeyer flask can maintain a desired temperature, sterile environment, and at low volumes (<1L), have sufficient oxygen mass transfer rates (2.0-20.0 h^{-1} at 100-160 rpm, 0.250-2.000 L flasks, with 0.125-1.200 L media) (Nikakhtari and Hill, 2005). However, drawbacks of flasks include lack of pH control and reduced aeration in scale-up—as aeration only occurs in the liquid-headspace interface which ratio with the volume decreases with larger volumes. Another consideration is if the liquid is too high up the neck of the flask then the aeration will be significantly reduced as well.

Stirred Tank Bioreactors

The stirred tank reactor (STR) is the most commonly used for microbial, single cell cultivations. It scales from 1 L to 10,000 L. Submerged impellers are the standard mixing practice for STRs. The aspect ratio and the impeller type are designed to establish to the mixing profile required for culturing microbial species. The two most common types of impellers are the radial-mixing Rushton type and axial-mixing marine type. With larger reactors, a mix of the two types of impellers is usually required to avoid stagnation zones and ensure good mixing. Baffles are

installed to prevent vortexing, a phenomena which reduces mixing. Aeration is usually provided by a ring sparger at the bottom of the tank; the diameter of the ring, the height position, and orifice size are all variable design parameters for spargers (Doran, 2013).

Oxygen mass transfer is well characterized in this type of reactor with literature suggesting the general form for predicting the volumetric mass transfer coefficient (Nikakhtari and Hill, 2005):

$$k_L a = A_0 * N^{A_1} * Q^{A_2} \qquad (2)$$

Where A_0, A_1, A_2 are empirically derived constants unique to each stirred tank bioreactor configuration, N is stirrer speed in rpm, and Q is aeration rate in L/min. A major drawback to STRs is similar to the shaker flask is that the energy required to properly mix the stirred tank bioreactor increases exponentially with volume. The second major drawback is increased shear stress at the impeller tip, τ_{tip}, which increases linearly with both impeller length and stirrer speed (Metzner and Otto, 1957):

$$\tau_{\text{tip}} \propto v_{tip} = r * N \qquad (3)$$

Shear stress is major concern because it can cause cell death by rupturing the cell membrane (Garcia-Ochoa and Gomez, 2009; Fontana and Silveira, 2012).

Airlift and Bubble Column Reactors

Airlift and Bubble Column bioreactors (ALR and BLR) have been thoroughly researched and are characterized similar to stirred tank bioreactors. Their ability to elicit growth curves and product yields have been verified for bacteria, actinomyectes, and filamentous mold (Gavrilescu and Roman, 1993; Alesieva and Peeva, 2000).

There is no shortage of literature describing the physical configurations and models for airlift and bubble column bioreactors. A schematic of airlift and bubble column bioreactors is presented in the methods section Figure 3.1. To discuss the general configuration briefly, a vertical column (high aspect ratio) is closed at the top and bottom with forced aeration at the bottom of the tank. The gas bubbles from the bottom of the tank rise to the top, creating a convective force for mixing and provides gas exchange (O_2, CO_2, CO, etc.) In conventional ALR and BLR, there is no mechanical mixing. However, configurations of ALRs and BLRs, which have added mechanical agitation or forced-liquid, have also been investigated (Christi and Jauregui-Haza, 2002; Yazdian et al., 2010).

Mixing time, θ, for ALRs and BLRs is increased with height according to the following proportionality.:

$$\theta \propto H^{1.7} \qquad (4)$$

This means during the scale up of ALRs and BLRs, non-homogenous zones will appear with noticeably reduced DO concentrations and will not be replenished until the next pass through the riser zone. However, bubble residence time is increased with larger heights, resulting in an increase in oxygen mass transfer rates. Conversely bubble size is also inversely proportional to oxygen mass transfer rates (Smart and Fowler, 1984).

The use of ALRs and BLRs as bioreactors has encompassed mostly plant-cell and bacterial cultures. However, fungal experiments have also been investigated. Srivastava and Kundu (1999) reported using an ALR to produce cephalosporin-C in a fungus, *Cephalosporium acremonium*. Their objective was to produce fungal pellets, as opposed to the conventional mycelia form. They cite enhanced mass transfer using

a bioparticle—Siran carrier. They reported $K_L a$ = 100 h^{-1} and 70 h^{-1} for Siran carrier and pellet modes, respectively.

The viscosity of the media is a great concern for ALRs and BLRs. As viscosity of the media increases, momentum transfer is reduced and energy dissipation is increased. ALRs and BLRs only source of mixing is the bubble rise velocity momentum transfer to the bulk liquid. While, operational power costs can be reduced by 17-64% in ALRs and BLRs over STRs, aeration must be sufficient not only to oxygenate the system but to have the system well mixed as well (Gavrilescu and Roman, 1993). To achieve good mixing, the use of a low viscosity liquid is recommended.

2.1.2 Solid-State Fermentation

Solid-state fermentation (SSF) is a less frequent strategy of fungal cultivation compared to submerged fermentation (SmF). SSF is conducted by seeding filamentous fungi onto a solid media, usually an agricultural residue such as cereal straw, which acts as physical support as well as a nutrient source. Three main configurations for this approach are the tray, packed bed, and rotating drum. The former two have no mechanical mixing and have low operating costs. Aeration is provided by either ambient air or forced air from the bottom of the reactor. The rotating drum has minimal energy requirements, as the bioreactor is turned over only periodically to mix the contents (Dhillon et al., 2013). SSF has no discernible liquid phase, however low levels of moisture are required for supporting the growth. (Robinson and Nigam, 2003).

Pandey et al. (2003) noted that filamentous fungi have been identified as the most successful organism to grown on solid media because of their ability to penetrate

through the pores of the substrate. SSF is considered the closest physical environment (i.e. damp wood) for a fungus, but large-scale practice difficult to obtain because of a lack of process control.

SSF in tray or packed bed configurations is an un-mixed, non-homogenous fermentation strategy, which leads to a loss of control over culture parameters such as pH, temperature, nutrient concentration, moisture, and oxygen transfer. High moisture causes low oxygen mass transfer, and is thought to promote anaerobic bacterial contamination. Heat generation during the exponential growth phase causes temperature build up in the SSF bioreactor. Increase temperature can denature products and hinder fungal growth. Measurement of bioreactor performance is also very difficult given the non-homogeneity of the culture and general lack of analytical techniques. Correlating headspace gas composition (CO_2 and O_2) is the most reliable tool for characterizing the growth (Pandey et al., 2003; Robinson and Nigam, 2003).

2.1.3 Other Fermentation Configurations

Coutte et al. (2010) used a bubbleless membrane bioreactor where a hollow fiber membrane (external and internal) was used for aeration in a conventional STR. An noteworthy advantage of bubbleless membrane bioreactors is that they do not foam.

Microbioreactors are a small scale alternative to the industry standard submerged fermentations. They are casually defined as fermentation in less than 1 mL. Materials for microbioreactors are usually plastic which allows for complete fluidic design into 'one piece'. The small scale and plastic materials also makes microbioreactors disposable which removes a requirement for sterilization and decreases setup time. Proper mixing is a concern in the microbioreactor as turbulent

mixing is difficult to achieve because of the small characteristic length. A lack of mixing does not mean a lack of gas exchange as it does in larger submerged fermentation. This is because at the small scale, membrane diffusion is sufficient for gas exchange (Schapper et al., 2009).

2.2 Airlift/Bubble Column Reactors and Pellet Morphology

Researchers reported the benefits of specific fungal pellet morphologies in terms of product yields and biomass growth (Aleksieva and Peeva, 2000; Srivastava and Kundu, 1999). Fungal pellet morphology is suitable for submerged fermentation because of the high surface area to volume ratio (Nitayavardhana, 2013). Fungal morphology can change from pellet to hairy-like which cause the fungus to grow over the impeller of a stirred tank reactor and other fittings, reducing mixing characteristics (Pilarek and Szewczyk, 2008). In ALR and BLR experiments conducted by Nitayavardhana et al. (2013), an aeration rate of 2.0 vvm resulted in a lower biomass yield coinciding with a morphological change to filamentous from pellet form.

The ability of ALRs and BLRs to combine oxygen mass transfer and bulk mixing with aeration allows them to provide a lower sheer environment while maintaining good aeration when compared to stirred tank reactors. Lower sheer is difficult to achieve in larger, mixed bioreactors due to the need for mixing. The shear stress cause by bubbles on the fluid and the walls on the fluid are very low compared to the impeller on the fluid in a stirred tank bioreactor (Wood and Thompson, 1986). However, since oxygen transfer is directly proportional to the aeration rate in ALRs and BLRs, oxygen transfer will be limited by the highest tolerated aeration rate which does not cause a morphological change.

ALR and BLR use also avoids the use of impellers, which can cause cell mortality (Coutte et al., 2010).

Ethanol concentrations can cause changes in morphology. Ethanol concentrations less than 3% do not adversely affect fungal biomass yields. At 5% ethanol concentrations, fungal pellets agglomerate causing mass transfer problems that inhibit growth (Nitayavardhana, 2013). In commercial vinasse, ethanol concentrations are lower due to higher recovery yields of ethanol (Nitayavardhana, 2013). This makes dilution not necessary for fungal fermentation on commercial vinasse. However, if the vinasse is non-diluted there will be unknown implications on the oxygen mass transfer properties due to a higher concentration of solutes in the vinasse.

2.3 Modes of Oxygen Transport

The first and primary mode of oxygen transport in fungal fermentations is through air bubbles forced through the liquid. The driving force of oxygen mass transfer is the concentration gradient, $(C^* - C)$ of Equation 1, between the saturated air and the dissolved oxygen in the bulk liquid. Oxygen gas must diffuse through each film in order to reach the bulk liquid—gas bulk to gas film to liquid film, to liquid bulk—as shown in Figure 2.1.

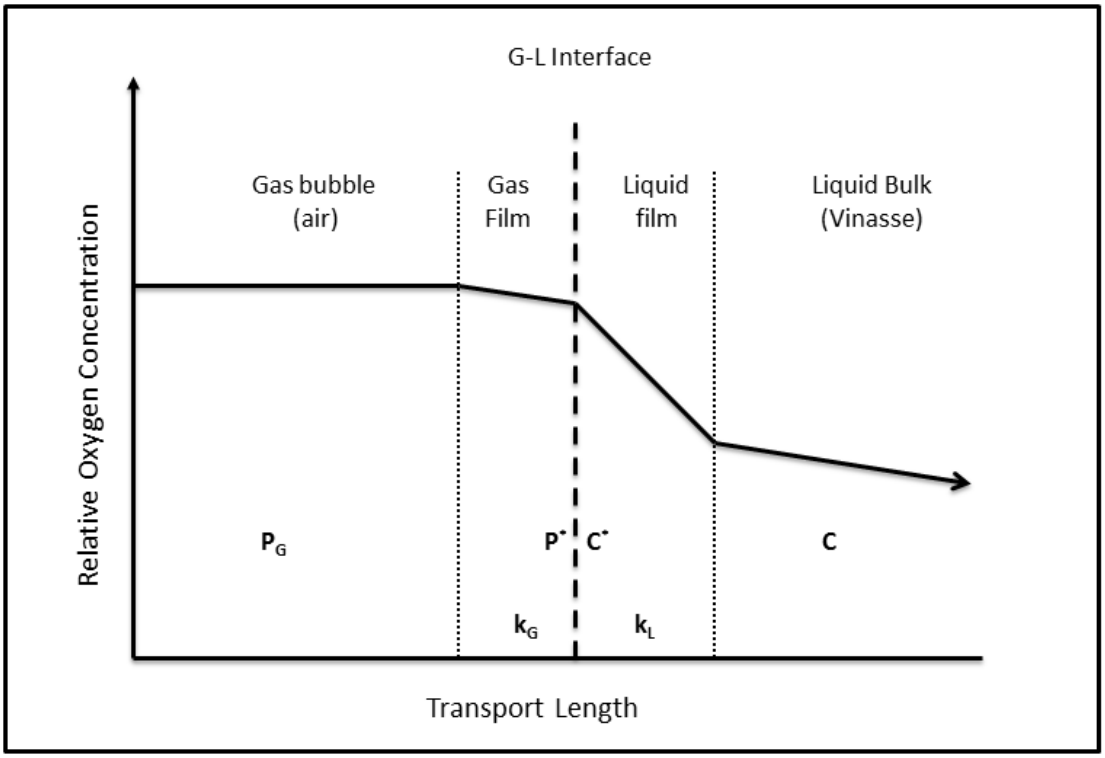


Figure 2.1 Oxygen concentration profile from air bubble to liquid media (adapted from Garcia-Ochoa and Gomez, 2009)

The mass transfer through the gas bulk and film is very fast compared to the mass diffusion through the liquid film and the liquid bulk. In engineering practice, the mass transfer rate is measured from the rate limiting step, the mass transfer through the liquid film, (i.e. $k_L(C^* - C)$). The assumption made for this calculation is that oxygen concentration at the gas-liquid interface equals the oxygen saturation concentration for the bulk liquid (determined by the maximal steady state oxygen concentration with no microbes present in the media) While this method lumps the gas film resistance, k_G, into k_L, there are studies which look at the two film resistances separately. For the gas mass transfer rate, the driving force is the difference between the gas bulk concentration and the gas-liquid interface concentration, $k_G(P_G - P^*)$. Where P^* and P_G are the interface and bulk gas concentrations, respectively (Garcia-Ochoa and Gomez, 2010).

Aeration through the head space-liquid interface can also contribute to the dissolved oxygen concentrations in the bulk media, but this effect is minimal. Christi and Jauregui-Haza (2002) reported that a maximum of 12% of the total oxygen found in solution originated from the head space of a reactor. The experiments of this research lumped together the gas bubble and surface aeration together to obtain the volumetric oxygen mass transfer coefficient,$k_L a$, for the total reactor configuration. The concern for this research is only to identify the oxygen mass transfer contribution by the entire system—sparger and head space—thus lumping together these two areas is defensible.

2.4 Volumetric Oxygen Mass Transfer Coefficient, $k_L a$, and its Measurement

The $k_L a$ can be empirically measured using the following differential equation represents the transient oxygen mass transfer in chemical reactions:

$$\frac{dC}{dt} = k_L a(C^* - C) \qquad (1)$$

Where $\frac{dC}{dt}$ is the change in liquid phase oxygen gas concentration over time, $k_L a$ is the volumetric mass transfer coefficient, C^* is the saturated liquid oxygen concentration, and C is the liquid oxygen concentration.

The volumetric oxygen mass transfer coefficient, $k_L a$, is a lumped parameter of the oxygen mass transfer coefficient, k_L, and the total volumetric gas-bubble surface area, a. $k_L a$ represents the proportionality coefficient that when multiplied by the driving force, (C^*- C), gives the oxygen mass transfer rate (OTR) from air bubble to liquid solution. There have been countless studies and proposed theories which try to analytically determine these two parameters and the numerous variables required for calculating $k_L a$ for ALRs and BLRs (Vasconcelos et al., 2003). The variables

which influence k_La be classified into four families of variables: physical properties, geometric parameters, operational conditions, and biomass concentration and type (Garcia-Ochoa and Gomez, 2009). It is clear that k_La is unique depending on bioreactor configuration and media combination. In addition, the transient nature of biological fermentations also causes physical properties to change which in turn affects the k_La value (i.e. K_La). Therefore, models that predict k_La are only applicable to small changes in the family of variables or mono-variant studies (i.e. changing the temperature only while leaving other variables like aeration rate constant).

Understanding the OTR of the bioreactor is important because it directly impacts the dissolved oxygen concentration in the bioreactor and ultimate the growth of the fungus.

If the geometric parameters (heights, diameter, sparger height, draft tube height, etc.) are fixed, including the sparger type. Then operational conditions that can be varied are temperature, aeration rate, and pH. Physical properties (viscosity, density, etc.) are dependent on the biomass and media type and will have transient values over the course of fermentation. Biomass weight and specific oxygen uptake rate will also be transient.

k_La can be determined by several different methods including chemical methods, sodium sulfite oxidation method, adsorption of CO_2, physical methods, and dynamic methods (Garcia-Ochoa and Gomez, 2009). By far the most widely used to determine k_La in ALRs and BLRs is the dynamic method where k_La measurement follows the following equation:

$$\frac{dC}{dt} = k_L a(C^* - C) - q_X * X \qquad (5)$$

Where, $q_X * X$ is the oxygen uptake rate.

For abiotic conditions $q_X * X = 0$ and the equation can be integrated to:

$$\ln (C^* - C) = k_L a * t \qquad (6)$$

A plot of ln (C) vs t will give a slope of $k_L a$.

When using polargraphic DO probes for measurements, the following equation corrects for probe response lag if the oxygen transfer rate is sufficiently high.

$$C = \frac{C^* - C_0}{1 - \tau_{RT} * k_L a} * \left[\tau_{RT} * k_L a * e^{-\frac{t}{\tau_{RT}}} - e^{-k_L a * t}\right] \qquad (7)$$

Where, τ is the electrode response time and C is the measured oxygen concentration. It can be seen from Eqn. 7 that the first term in brackets is becomes negligible as t increases and for smaller values of τ_{RT}(i.e.$e^{-\frac{t}{\tau_{RT}}} \rightarrow 0$).

The electrode response time, τ_{RT}, is used to determine if this correction equation is required. τ_{RT} is the experimentally recorded time for a probe to measure a step change in concentration up to 63.2% of the final value (Tribe et al., 1995). If τ is of the same order of magnitude as $\frac{1}{k_L a}$ then the correction equation should be used (Van't Riet, 1979). For example, if the $k_L a = 0.01\ s^{-1}$ then $\frac{1}{k_L a}$ = 100 s which is almost two orders of magnitude greater than the typical response time in most cases: τ ≈ 5-6 s. ALRs and BLRs with only aeration and no mechanical mixing produce $k_L a$ values between 0.0027 and 0.0277 s^{-1}, which corresponds to $1/k_L a$ values from 360 and 60, respectively. Thus, for $k_L a \leq 0.0277\ s^{-1}$, probe response lag can be neglected. The correction equation can also be avoided if the first 30% of DO concentration data is truncated (Merchuk et al., 1990).

Measurement of $k_L a$ with respiring biological components active in the media is more involved. When reporting the $k_L a$ of a biological system, the volumetric mass transfer coefficient is written with a capital K (i.e. $K_L a$) to signify the presence of third dispersed phase (in this case the fungus). When the oxygen concentration cannot be increased from low levels, such as when the fungal OUR is greater than the OTR, a modification to the dynamic method has been developed. This method uses varying concentrations of oxygen in the inlet streams (i.e. air and pure oxygen), which will produce two pseudo steady-states for which $K_L a$ can be determined (Garcia-Ochoa and Gomez, 2009). This method is useful for on-line $K_L a$ measurements when microbes present in the bioreactor cannot tolerate low oxygen levels, or the normal aeration cannot sustain a detectable DO concentration (i.e. DO = 0%)

Another method to determine the $K_L a$ in bioactive fermentation is to determine a mass balance of the inlet and outlet air streams ($F_{O_2}^{in}$, $F_{O_2}^{out}$) near steady state ($\frac{dC_{O_2}}{dt} = 0$). Any oxygen loss in the air therefore is contributed to transfer to the media or oxygen uptake by the organism. A third probe in the liquid can determine the steady-state liquid oxygen concentration (C_L). Finally:

$$K_L a = \frac{F_{O_2}^{in} - F_{O_2}^{out}}{V * (C^* - C)} \qquad (8)$$

Where, V is volume of the reactor. For very small reactors where there are minute changes in $F_{O_2}^{in}$ and $F_{O_2}^{out}$, very sensitive oxygen probe for the gas streams is required. On the other hand, if the liquid volume is large and a significant amount of oxygen is transferred to the liquid media from the bubble, less sensitive equipment is needed.

There are even more developed chemical and physical methods for determining $k_L a$ outside the conventional methods. Pedersen et al. (1994) also reported using radioisotopes for $k_L a$measurement. However, these methods are undesirable for fungal growth experiments because of the toxic effect on fungal growth and unknown reactions with solutes in vinasse.

2.5 Modeling the volumetric oxygen mass transfer coefficient

Numerous authors have proposed models for predicting $k_L a$ in ALRs and BLRs (Kantarci et al, 2005; Garcia-Ochoa and Gomez, 2009). Most models require the knowledge of many physical and operation parameters including: Diffusivity, viscosity, density, superficial gas velocity, and gas hold-up ($D, \mu, \rho, v, \varepsilon$). Garcia-Ochoa and Gomez (2009) presented the general form of predicting $k_L a$ by taking the common terms from multiple researchers' empirical equations for ALRs and BLRs:

$$k_L a = c * v_s^a * \mu_a^b \qquad (9)$$

Where, C, a, b are empirical constants, $v_s$is superficial gas velocity, and μ_a is apparent viscosity. Values for C and a in pure water have been determined: $c = 0.47, a = 0.82$ (Deckwer et al., 1974, 1983). When the fluid is non-Newtonian, apparent viscosity influences the calculation. Godbole et al. (1984) reported values between -0.8 and -1 for the exponent b. Geometric properties, such as height and diameter of the bioreactor, have less influence on prediction of $k_L a$ in ALRs and BLRs because they do not affect the boundary layer between the air bubble and the liquid stream.

2.6 Biological Enhancement Factor

The biological enhancement factor, E, is a phenomenon that either increases or decreases $k_L a$ because of a microorganism physical presence and oxygen uptake rate.

E is defined as the ratio of oxygen mass transfer rate in the presence of cells $K_L a$ to the oxygen mass transfer rate without cells, $k_L a$.

$$E = \frac{K_L a}{k_L a} \qquad (10)$$

When the cell concentration is high enough, the bubbles do not interact with the bulk liquid velocity. Instead, they are exposed to the microorganism first (along with a thin surfactant layer) and the film resistance changes due to this phenomenon (Garcia-Ochoa et al., 2010). A model predicting E was proposed by Garcia-Ochoa and Gomez (2005):

$$E = \left[1 + \frac{q_X C_{X,m}}{2D_m(C^* - C)} * \left(1 + 2 * \frac{z_L D_m}{z_m D_L} + \frac{2z_L^2}{3z_m^2}\right) + \frac{1}{3}\frac{q_X C_{X,L} z_L^2}{2D_L(C^* - C_L)}\right] * \left[\frac{\frac{z_L}{D_L}}{\sum_i \frac{z_i}{D_i}}\right] \quad (11)$$

Where $C_{x,m}$ and $C_{x,L}$ are the cell concentrations in the monolayer and stagnant liquid layer, respectively. Where q_{O_2} is the specific oxygen uptake rate and C_L is the oxygen concentration in the liquid. z_i and D_i are the film thicknesses and diffusivities, respectively. This model predicts that E will increase for increases in both cell concentrations and cell specific oxygen uptake rates. The value of *E* has been reported to range from 0.9 to 2 for bacterial cultures. (Garcia-Ochoa and Gomez, 2005). However no fungal enhancement factors have been reported.

2.7 Volumetric Surface Area, Air Bubble Size, Gas Bubble Velocity, Kinematic Viscosity, and Diffusivity

Many of the physical properties are functions of the operational parameters and other physical properties. For example if you decrease your bubble size you will increase your surface area and increase your gas hold up. Bubble size is a function of sparger

type, aeration rate, physical properties of the broth, and biomass properties. Bubble size is very difficult to maintain uniform throughout a fungal fermentations, however average bubble size equations have been deemed sufficient for calculating the volumetric surface area, a. Smaller bubbles have an increased surface area to volume ratio, which enhances the exposed interface between gas and liquid. Reducing bubble size will generally lead to increases in a and thus increases in $k_L a$ in pure water. Kantarci et al (2005) proposed an equation relating the superficial area to gas hold up and bubble diameter:

$$a_s = \frac{6\varepsilon}{d_s} \qquad (12)$$

For solutions with contaminants or substances that can adhere to the gas-liquid monolayer, Vasconcelos et al. (2003) hypothesized and tested that smaller bubble radii can decrease k_L from the resistance caused by rigid interfaces at the rear of rising bubbles. Thus, when there is physical blocking in the media, larger bubbles have greater k_L due to a more mobile interface with the liquid. Vasconcelos et al. (2003) presented theoretical equations for $k_L^{ridged} and\ k_L^{mobile}$:

$$k_L^{ridged} = \frac{0.6\sqrt{\frac{v_S}{d}}D^{\frac{2}{3}}}{\nu^{\frac{1}{6}}} \qquad (13)$$

$$k_L^{mobile} = 1.13\sqrt{\frac{v_S}{d}}D^{\frac{1}{2}} \qquad (14)$$

From these equations, the effects of slip velocity, (v_S), kinematic viscosity,(ν), bubble diameter, (d), and gas diffusivity, (D), can be observed. Slip velocity, (v_S) is defined as $v_S = \frac{v_G}{\varepsilon} - u_L$, where v_G and u_L are superficial air bubble velocity and free stream liquid velocity, respectively.

Although bubble size and film resistance is an important parameter for fungal fermentations, calculating the lumped parameter $k_L a$ does not require calculating the two individual separately. On the other hand, many experiments will calculate the lumped parameter first, estimate one of the parameters and solve for the last unknown (i.e. $k_L = \frac{k_L a}{a}$). The importance of calculating the individual parameter *a* is that researchers can pinpoint the changes in $k_L a$ to either k_L or a. For example if *a* can be estimated, a researcher can test how a bioreactor component such as decreased sparger orifice affects k_L, independently from *a*.

2.8 Gas Hold Up

Gas hold up is a measurement of the colloidal entrapment of gas within the liquid phase. This entrapment of gas adds volume to the bioreactor working volume and is defined generally as $\varepsilon = \frac{\Delta V}{V_o + \Delta V}$, where ΔV is the volume change after aeration and V_o is the volume without aeration. Increases in gas hold up are due to many physical, geometric, and operational conditions and can lead to increases in $k_L a$ due to increases in interfacial time as well as volumetric interfacial area, a. For increases in aeration rates, gas hold-up will necessary increase along the equation (Garcia-Calvo and Leton, 1991):

$$\varepsilon = \frac{v_s}{U_s + \frac{1}{2} V_{LC} + \bar{V}_{LR}} \qquad (15)$$

Where U_s, V_{LC}, and $\bar{V}_{LR}$ are terminal rise velocity of the bubble, average velocity in the core region, and average linear velocity, respectively. The consequence of increased gas hold up is increased $k_L a$, by means of an increase in volumetric interfacial area (section 2.7).

We have discussed what k_La is, how to measure k_La, parameters that affect k_La. Now we will discuss problems that occur during bioreactor operation that may influence, distort, under-represent or over-represent the k_La.

2.9 Foaming

Foaming is a primary concern for fungal fermentation in ALRs and BLRs. Foaming can lead to lower culture yield due to the organism being entrapped in the foam bubbles as well as increase risk of contamination. Junker (2007) outlined many adverse consequences due to foaming, such as lower product yield and damage to instruments of the reactor.

The strongest way to mitigate foam is to modify the media being used, most often by dilution because dilution lowers the surface tension--the cause of bubble formation. There are also antifoam reagents and foam breaker methods. However, the addition of antifoams can be toxic to fungal species and foam breaking systems add capital and operation costs to bioreactors (Junker, 2007).

On the other hand, there is a benefit to foaming. Foaming can increase oxygen mass transfer by increasing the volumetric area at the surface, allowing for more headspace oxygen mass transfer(Junker, 2007). However foam that entraps the organism must be avoided.

Higher electrolyte concentrations and lower bubble rise velocities contribute to lower bubble coalescence at the liquids top surface (Del Castillo et al., 2011). Lower bubble coalescence will increase foaming, gas hold up, and the volumetric mass transfer coefficient (k_La).

2.10 Yeast Contamination of Vinasse

Nitayavardhana et al. (2013) discussed yeast contamination of the fungal cultivation on vinasse in ALR and BLR (2.5 L) experiments. They showed that either vinasse was not completely sterilized or the yeast was reactivated after 8 hours of fermentation. A maximal soluble oxygen uptake rate of the contamination was a maximum at 24 hours. The yeast cell activation and growth competes for oxygen with the fungus and can transiently change the K_La and biological enhancement factor, E. It can also be inferred that yeast cells also increase the viscosity and physical blocking of oxygen mass transfer in vinasse media, reducing K_La.

Junker et al. (2006) outlines sources of contaminations, as well as design and procedures for minimizing contamination at the pilot plant scale. Factors that can increase contamination likeliness are: temperature in 20-40°C range, pH of 5, rich complex medium with high nutrients, and high pre-sterilization burden (due to yeast growth for ethanol fermentation). They suggest nine methods for reducing contamination, but many would also kill the fungal species or are not applicable to the industrial fungal processing of vinasse. Two methods to combat contamination would be low dissolved oxygen and addition of antibiotics. Both are unfeasible because 1. lowering DO would cause the aerobic fungal cultivation to collapse 2. Antibiotics are added costs that cannot be sustained by a co-product generation unit process. In any fermentation, contamination will lead lower growth rates and fungal yields by a competition for nutrients and oxygen. Best efforts should be made to reduce contamination prior to and during fermentation.

2.11 Fungal Growth for Industrial Fermentations

The estimated value of fungal biomass as a feed ingredient is $515-1,537 per metric ton (USDA, 2012). With fungal biomass yield about 3.79 g/L and 40 billion gallons of vinasse available through Brazil's sugarcane ethanol production (US DOE, 2006; Nitayavardhana et al., 2013;), the estimated gross value of a fungal fermentation unit process could be $11-32 billion dollars.

$$40 * 10^9\ gal * 3.785 \frac{L}{gal} * 3.79\ \frac{g\ biomass}{L} * \frac{1\ kg}{1000\ g} * \frac{\$515\ to\ 1537}{1000\ kg} = \$11\ to\ 32\ billion$$

In order to realize this unit process, the industrial fermentation must efficient and economical. Lowering the operational costs of fungal fermentation at the industrial scale should be a priority. The oxygen mass transfer rate is controlled by the aeration rate which is the only operation costs other than pumping of liquids in airlift and bubble column bioreactors. Thus an investigation into ways to reduce aeration will lead to a more efficient and less costly fungal fermentation. For fungal fermentation on a vinasse substrate, there have been no studies which look at the oxygen mass transfer properties in this system. The OTR is governed by the volumetric oxygen mass transfer coefficient, $k_L a$. Insight into the OTR given by measuring the $k_L a$ will allow aeration to be tailored to the oxygen uptake rate of the fungus. Maintaining the minimum aeration for maximal yields will lower operational costs and provide maximal profit for a fungal fermentation unit process in ethanol production and vinasse disposal. Optimal use of vinasse substrate will also reduce the environmental impact when the waste water is released into the water table.

CHAPTER 3 MATERIALS AND METHODS

3.1 Sugarcane Vinasse Preparation

The sugarcane vinasse was prepared in the laboratory by ethanol fermentation of sugarcane syrup by yeast followed by ethanol recovery.

3.1.1 Ethanol Fermentation

Sugarcane syrup was obtained fresh from Hawaiian Commercial and Sugar Company (Puunene, Hawaii, USA) and stored at -10 °C. The syrup was diluted with distilled water to a sugar concentration of 15 g sucrose/g solution (°brix) and used as a substrate for ethanol fermentation by yeast. The pH was adjusted to 4.00 ± 0.05 using 10% (w/v) NaOH or 10% (v/v) H_2SO_4. The media was sterilized in an autoclave (HICLAVE™ HVE-50, Hirayama, Japan) for 20 min at 121 °C.

A 7.5 L stirred tank bioreactor with control unit (BioFlo 110, New Brunswick Scientific Co., Inc., NJ, USA) was used for the ethanol fermentation. The working volume was 5.50 L sugarcane syrup media. The bioreactor was first washed with soap and water then all surfaces were sterilized with 70% (v/v) ethanol. A 1 g/L inoculum was prepared by adding 5.50 g Dry Brewer's Yeast, *Saccharomyces cerevisiae,* (Muntons Plc., Cedars Maltings, Stowmarket, Suffolt, IP14 2AG, England) to 20 mL deionized water. The yeast inoculum was reactivated in an incubator shaker (Inova™4 230 and Excella E25, New Brunswick Scientific Co., Inc., NJ, USA) for 15 min at 150 rpm at 37 °C. The reactivated yeast was inoculated to the stirred tank bioreactor at the 0^{th} hour with operating conditions of 30 °C, agitation speed of 300 rpm, and aeration rate of 2.5 L min. The pH was maintained at 4.0 using 2% (w/v) NaOH or 2% (v/v) H_2SO_4. The aerobic fermentation time was 6 hours to allow the yeast

population to acclimate and grow. Anaerobic conditions were implemented after the 6th hour. Aeration was removed and the agitation was reduced to 100 rpm; pH and temperature remained constant. The fermentation beer was collected after 72 hours.

3.1.2 Ethanol Recovery

Distillation of ethanol from the yeast fermentation broth was carried out using a rotary evaporator (Rotavapor R-215, Buchi Labortechnik AG, Flawil, Switzerland). The operating conditions were 50 °C, 120 rpm for 30 min. Ethanol recovery was done using ~1 L of fermentation broth at a time. Vacuum pump was used to maintain a pressure of 96 kPa (Nitayavardhana et al., 2013). Recovered ethanol/water was discarded and the remaining fermentation broth called vinasse was sterilized via autoclaved at 121°C for 20 min and stored at 4 °C until further use in airlift and bubble column reactor experiments.

3.2 Airlift and Bubble Column Bioreactor Fermentation

3.2.1 Fungal Spores

An initial fungal spore suspension of *Rhizopus microsporus* (var. *oligosporus*) was obtained from American Type Culture Collection (ATCC # 22959, Rockville, MD, USA). A stock spore suspension was created by propagating the fungus on sterile potato dextrose agar Petri dishes (Difco Laboratories, Sparks, MD, USA), and the plates were incubated at 24 °C for 5-7 days; until black fungal spores were formed as per protocol reported by Nitayavardhana et al. (2013). Plated fungal spores were harvested with a solution of 0.1% (w/v) peptone and 0.2% (v/v) Tween 80 (Fisher Scientific, Fair Lawn, NJ, USA). Then, all the spores harvested were pooled together to maintain a homogeneous spore count. Glycerol was added to the harvested spores

in a 20% (v/v) before storage at -20 °C. A 5.28 x 10^6 spores/ml concentration was obtained via hemocytometer (Hausser Scientific, Horsham, PA, USA).

3.2.2 Fungal Starter

A 500 mL, 0.2 % (v/v) fungal spore/yeast mold (YM broth) solution was prepared for inoculating the bioreactor with fungus. YM broth was prepared via Difco following manufacturer's protocols. Briefly, 10.5 g YM Broth (Difco Laboratories, Sparks, MD, USA) was added to 500 mL deionized water in a 1 L Erlenmeyer flask. For sterilization, the YM broth was autoclaved for 20 min at 121 °C. After the YM broth cooled to room temperature, 1 mL stock fungal spore suspension (section 3.2.1) was added to the sterile media. The fungal starter was incubated in an incubator shaker for 72 hours at 37 °C and 150 rpm.

3.2.3 Bioreactor Dimensions and Materials

A 3.5 L cylindrical column bioreactor was fabricated in-house from clear acrylic 0.5 cm thickness. The height of the reactor was 40 cm from base to head. The inner diameter was 14 cm. For airlift experiments, a circular draft tube (0.5 cm acrylic) was inserted to the bioreactor at 3.5 cm from the base and 17 cm from the head. The draft tube was 16 cm in height, had a 9 cm inner diameter, and 10 cm outer diameter. The resulting riser/downcomer area ratio was 0.96. For bubble column configuration, the draft tube was removed from the same airlift reactor. The air sparger was located at the center of the base, protruding 3.75 cm above the base, with four evenly spaced air diffusers. The air diffusers were Lee's Discard-A-Stone, Fine (Lee's Aquarium & Pet Products, San Marcos, CA, USA). Air was supplied by a liner air pump (Super Pond, Kennewick, WA, USA) and regulated with a 65 mm rotameter (Omega Engineering Inc., Stamford, CT, USA). Inlet air was filtered through a 0.1

μm pore size polytetrafluoroethylene membrane (Whatman, Florham Park, NJ, USA). The pH was measured with a pH probe (#405-DPAS, Mettler Toledo-Ingold Inc., Bedford MA, USA), controlled by Eutech Instruments pH 200 Series controller (Cole-Parmer Instrument Company , Vernon Hills, IL, USA) and acid and base was pumped by a Pulsafeeder® (Punta Gorda, FL, USA). The temperature was maintained by submerging the bioreactor in a temperature controlled water bath. Figure 3.1 shows a schematic of the bioreactor setup. The superficial gas velocity is calculated below for 1.5 vvm.

$$\text{Gas flow rate } \left(\frac{\text{L}}{\text{min}}\right) = 1.5\ L/L/\text{min} * 2.5\ L = 3.75 \frac{L}{min}$$

$$v_s = \frac{3.75\ L}{min} * \frac{1\ min}{60\ s} * \frac{1000\ cm^3}{1\ L} \frac{1}{\pi \left(\frac{14\ cm}{2}\right)^2} * \frac{1\ m}{100\ cm} = 0.00406\ m/s$$

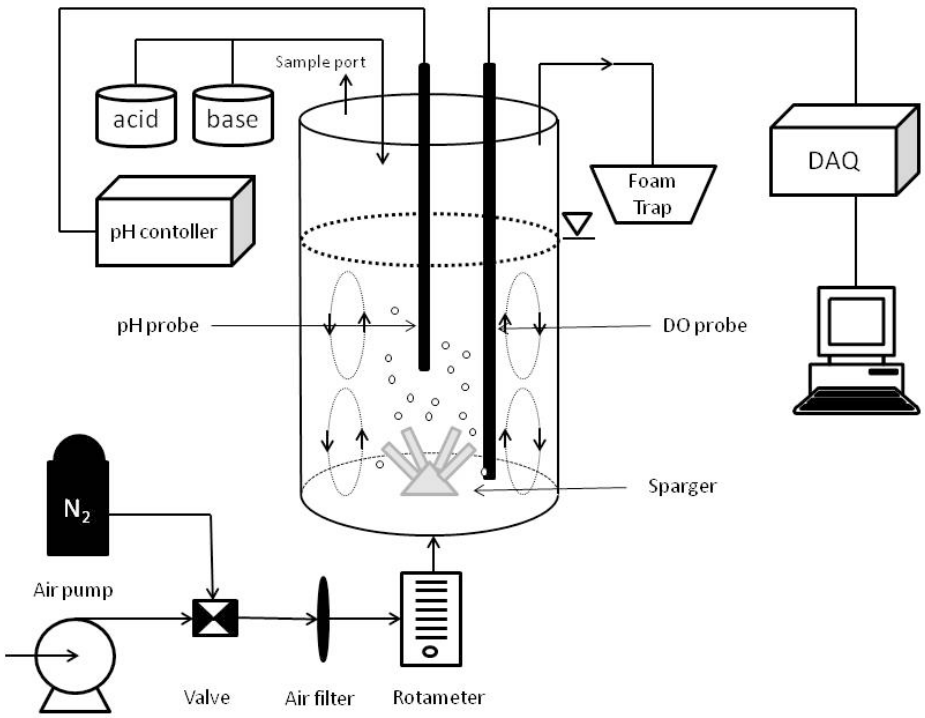


Figure 3.1 Bioreactor schematic in bubble column configuration

3.2.4 Fungal Culture on Vinasse Substrate (with-fungus)

The working volume of the fungal fermentation was 2.5 L. A 2.0 L 75% (v/v) vinasse media was prepared by diluting 1.5 L vinasse with 0.5 L distilled water. The vinasse media was sterilized in an autoclave and added to the bioreactor. The 500 ml fungal starter (section 3.2.2) was added directly (residual YM broth and fungal pellets) to the bioreactor. The pH was brought to 5.0 with 10% (w/v) NaOH and maintained by 2% H_2SO_4 or NaOH solutions. Temperature was maintained at 30° C. The reactor working volume was constant. No nutrient supplementation was added. Airflow was 1.0 vvm until $K_L a$ measurements were taken.

$K_L a$ was measured at aeration rates of 0.5, 0.75, 1.0, 1.25, 1.5, 2.0 vvm. Each $K_L a$ measurement was done successively, one after another. Immediately after $K_L a$ measurements were done, the reactor was harvested, and a dry weight of fungal biomass was recorded. Different cultivation times were used (~12, ~ 24, ~48 hours) and resulted in varying, though not correlated, biomass dry weights. This allowed the experiment to compare the changes in $K_L a$ versus final biomass dry weight.

The fungal biomass dry weight was used as indicator of fungal biomass in the reactor as the fungus is sponge-like and measurements of wet weight would be inaccurate. The fungal biomass was harvested (after $K_L a$ measurements) by passing the fermentation broth over a #60 sieve (VWR, Radnor, PA, USA). The fungal biomass was dried at 70 °C for 48 hours or until a constant weight was achieved.

3.2.5 Water-only Operation

The working volume for water only experiments was 2.5 L. All initial factors (pH, temperature, sterility) but one were identical to the ‘With-fungus’ (section 3.2.4)

experiments. Instead of vinasse media, 2.5 L distilled water was used as the control media for determining baseline oxygen mass transfer properties of the bioreactor configuration.

3.2.6 Vinasse-only Operation

The working volume of vinasse-only experiments was 2.5 L. All initial factors (pH, temperature, sterility) but one were identical to the 'With-fungus' (section 3.2.4) experiments. The fungal starter was replaced with 500 mL sterilized deionized (DI) water. No fungal harvest was needed for these experiments. No maintenance of pH was required as well.

3.3 Analytical Methods

3.3.1 Dissolved Oxygen Measurement and Volumetric Mass Transfer Coefficient Determination

The dissolved oxygen was measured with a polargraphic dissolved oxygen probe (P0720-6283, Mettler Toledo-Ingold Inc., Bedford, MA, USA). The probe was placed off-center of the bioreactor, 3 cm above the base to prevent bubble fouling the membrane. The analog dissolved oxygen percent (DO) was recorded using the BioFlo 110 controller and the signal was converted to a digital format using a data acquisition card (Measurement Computing Corporation, Norton, MA, USA) with a sampling rate of 1 Hz.

To determine $k_L a$ in water-only and vinasse experiments according the dynamic method (Section 2.4), a DO curve was created (Figure 4.3). The DO was reduced to a low value (0-40%) using nitrogen gas sparged through the air diffuser. The air was introduced at the low DO level simultaneously with the closure of the

nitrogen supply (using a T joint and ball valves). The DO was allowed to reach its saturation point and the experiment was repeated to obtain n≥3.

3.3.2 Volumetric Mass Transfer Coefficient Calculations

The DO (% saturation) curves were imported as delimited values into Excel 2007 (Microsoft Corporation, Redmond, WA, USA). For water-only and vinasse-only experiments, a scatter plot of $\ln(1 - DO)$ vs. time (s) was created to linearize the curve according to Eqn. 6. To take into account the oxygen uptake rate by the fungus, the maximum oxygen concentration, C_{max}, replaces C^* in Eqn. 6. For with-fungus experiments, a scatter plot of $ln(C_{max} - DO)$ vs time (s) was created to linearize the curve according to dynamic method for $k_L a$ calculations. A linear regression line was fitted in Excel 2007 and the slope = $-k_L a$ for each case. $k_L a$ was recorded in s^{-1} and converted to h^{-1} by multiplying by 3600 $\frac{s}{h}$.

For water-only, ALR and BLR configurations,$k_L a$ values were fit with a power regression according to the model . The coefficient and exponent was found for a modified Eqn. 9 (Section 2.5) which we have lumped the apparent viscosity term into the coefficient, *c*, since there was no physical changes to the media during these measurements:

$$k_L a = c * vvm^a \qquad (16)$$

Where c is a proportionality constant with units $\frac{min}{h}$, vvm is the aeration rate in $\frac{\frac{L}{L}}{min}$ and a is empirical constant.

3.3.3 Statistical Analysis and Measurement Error

Sample variance was checked for consistency with an F-test in order to assume equal variance for the t-test. One-way analyses of variance, t-test assuming equal variance was performed on between group measurements. A confidence interval of 0.05 was used. Excel 2007 Analysis Toolpack Add-in (Microsoft Corporation, Redmond, WA, USA) was used for this analysis.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Rationale

Vinasse is a highly complex medium with many organic and inorganic solutes (Nitayavardhana and Khanal, 2010). Complex solutes in solution can have a detrimental effect on bioreactor properties such as viscosity, density, gas hold up, foaming, and additional resistance in the liquid film resistance. In order to determine the effects of these solutes on oxygen mass transfer, baseline $k_L a$ values for water-only and vinasse-only fermentations was needed for comparison. A water-only control curve of $k_L a$ values at varying aeration rates will prove useful when determining if further studies on solute effects on $k_L a$ are warranted.

$k_L a$ values for vinasse-only ALR and BLR configurations are also needed to provide engineers with an idea of the mass transfer properties to expect when vinasse is the chosen media. Different aeration rates will promote different $k_L a$ values which can be tailored to the oxygen uptake rate of the fungus during fermentation.

Fungal growth within the bioreactor adds additional complexity to the medium and oxygen mass transfer properties. The results from measuring $K_L a$ when fungus is growing in the reactor will give us information regarding actual effect of live growth on oxygen mass transfer properties.

The experimental design was to look at abiotic and fungal systems using ALR and BLR configurations with water and vinasse media. However, there are no results for the airlift configuration with vinasse media because there was bioreactor fowling during the fungal fermentation in the ALR configuration with vinasse media. In the 2.5 L airlift bioreactor, it is nearly impossible to prevent plugging of downcomer area

when fungal biomass agglomerates. Figure 4.1 is an example of plugged airlift bioreactor. Only a larger area for passage would allow for the agglomerated fungus to pass through. Higher shear rates also cause a more hairy like protrusion of the fungal pellet, which was observed visually. During bubble column operation, the pellets were able to grow in a denser pellet which prevents fungal biomass agglomeration.

Figure 4.1 Plugged airlift bioreactor

4.2 Dynamic Method Data and Volumetric Mass Transfer Coefficient Calculations

Dynamic method measurement of DO versus time curves was obtained for $k_L a$ calculations (Section 3.3.2). At least three peaks were obtained for each configuration. The configurations obtained are summarized in Figure 4.2. Fungal biomass fouling in the space between the side wall and draft tube is consistent with the findings of Nitayavardhana et al. (2013). Fungal biomass was also found to foul the DO probe by attaching at the tip, preventing a steady stream of bulk liquid to pass over the membrane. A consequence of reactor plugging and DO probe fouling is that no airlift data for with-fungus and vinasse-only are presented for this research. There are methods discussed to combat this issue in Chapter 6.

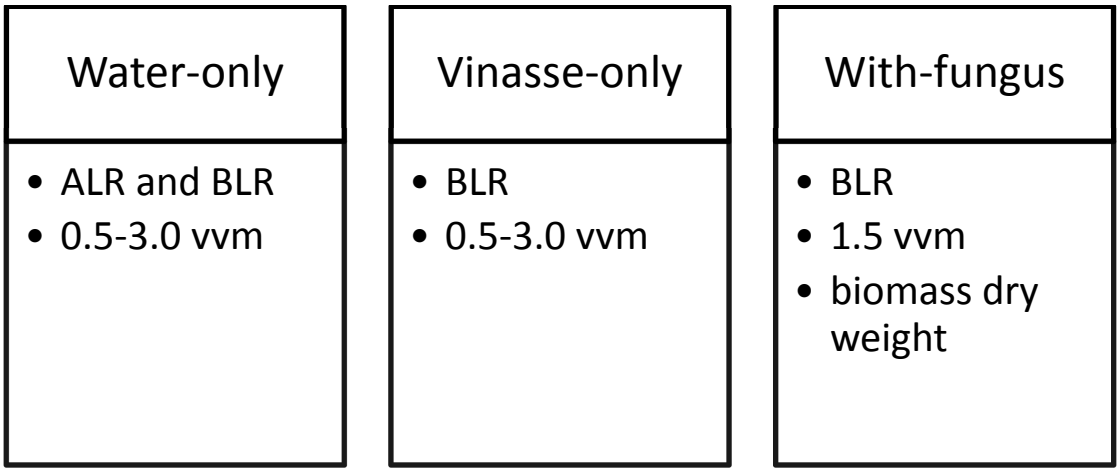


Figure 4.2 Summary of volumetric oxygen mass transfer coefficient data to be presented for media, bioreactor configuration, and aeration rate.

Dissolved oxygen versus time plots were obtained from the ALR and BLR configurations at varying aerations rates for water-only. A typical DO versus time curve is presented in Figure 4.3. The downward sloped data were obtained during nitrogen sparging and the upward sloping data were obtained during air sparging.

Next, at least 30 data points were used to plot ln(1-DO) versus time. A linear regression gave the values of $k_L a$. A typical linear curve for $k_L a$ calculations is presented in Figure 4.4. The $k_L a$ and $K_L a$ values for all experiments are found in Tables 4.1-4.5.

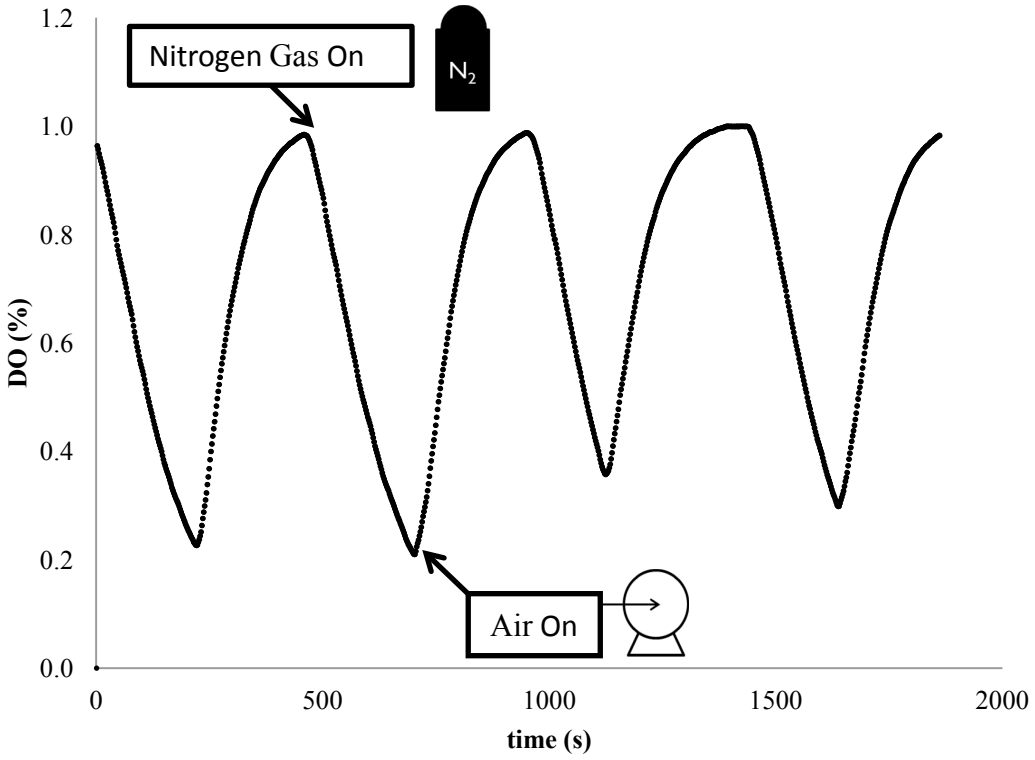


Figure 4.3 Dissolved oxygen vs. time curve obtained from bioreactor experiment. Conditions: water-only, bubble column configuration, aeration rate 1.5 vvm, sample rate 1 Hz

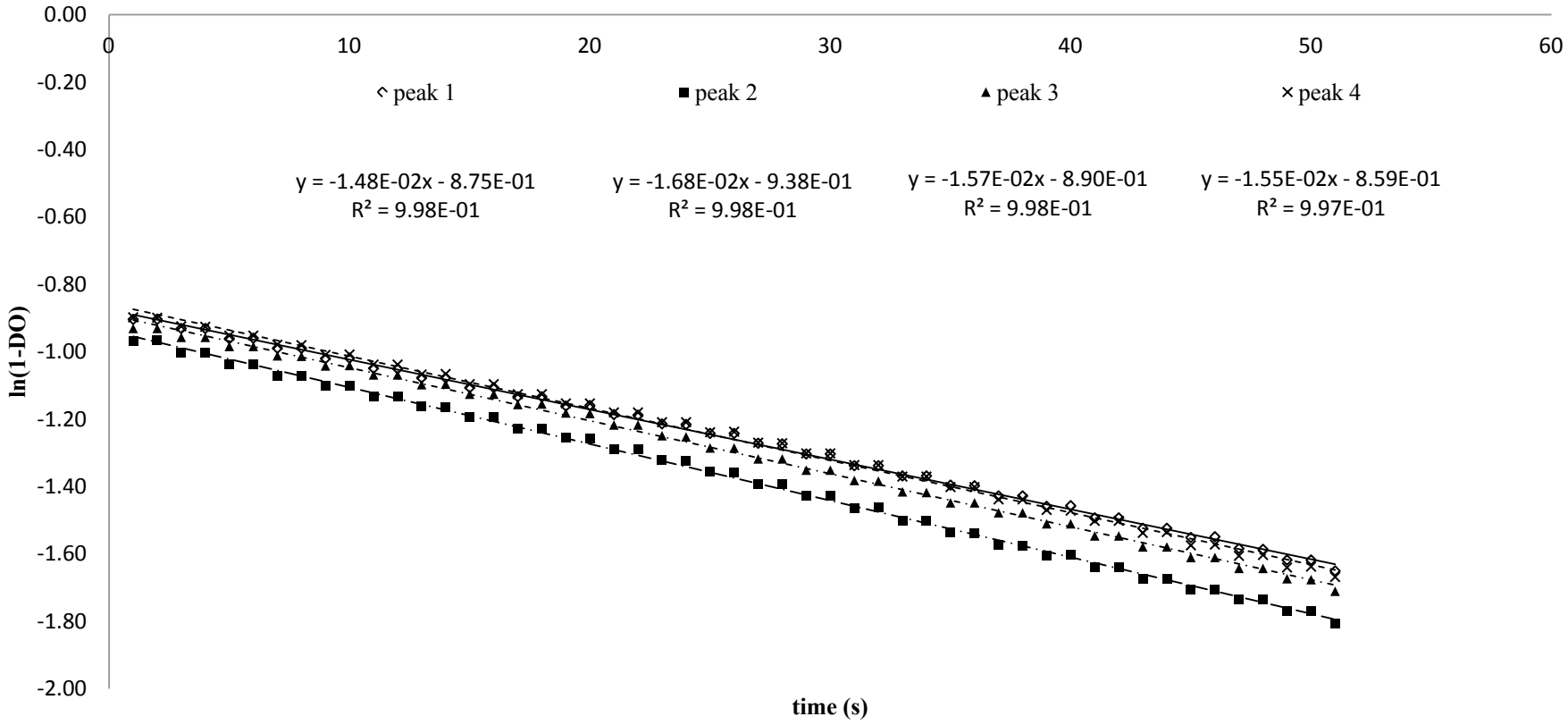


Figure 4.4 Typical $ln(1 - DO)$ vs. time curve to obtain $slope = -k_L a$. Conditions: water-only, bubble column configuration, aeration rate 1.5 vvm, sample rate 1 Hz

Table 4.1 Volumetric oxygen mass transfer coefficient values for water-only airlift bioreactor experiments

Aeration Rate (vvm)	$k_L a$ (h^{-1})	Mean (h^{-1})	Std. Dev. (h^{-1})
0.5	19.5	17.8	1.5
0.5	17.4		
0.5	16.6		
0.8	34.9	32.6	2.0
0.8	31.4		
0.8	31.4		
1.0	41.0	39.6	1.4
1.0	39.6		
1.0	38.2		
1.3	47.9	46.8	1.0
1.3	46.4		
1.3	46.1		
1.5	56.5	53.0	2.8
1.5	53.6		
1.5	51.8		
1.5	50.0		
2.0	70.6	71.9	2.9
2.0	69.8		
2.0	75.2		
3.0	104.8	100.8	4.2
3.0	101.2		
3.0	96.5		

Table 4.2 Volumetric oxygen mass transfer coefficient values values for water-only bubble column experiments

Aeration Rate (vvm)	$k_L a$ (h^{-1})	Mean (h^{-1})	Std. Dev. (h^{-1})
0.5	18.0	18.6	0.6
0.5	18.8		
0.5	19.1		
0.8	31.5	32.0	0.8
0.8	31.7		
0.8	32.9		
1.0	43.6	43.3	0.7
1.0	43.9		
1.0	42.5		
1.3	46.4	45.8	0.5
1.3	45.7		
1.3	45.4		
1.5	55.8	56.5	3.0
1.5	56.5		
1.5	53.3		
1.5	60.5		
2.0	72.4	72.4	2.5
2.0	74.9		
2.0	69.8		
3.0	77.8	79.4	2.2
3.0	82.4		
3.0	79.6		
3.0	77.8		

Table 4.3 Volumetric oxygen mass transfer coefficient values values for vinasse-only bubble column experiments

Aeration Rate (vvm)	$k_L a$ (h^{-1})	Mean (h^{-1})	Std. Dev. (h^{-1})
0.5	10.2	10.1	0.2
0.5	9.9		
0.5	10.2		
0.8	13.4	13.2	0.3
0.8	13.3		
0.8	12.8		
1.0	19.9	20.7	0.8
1.0	20.8		
1.0	21.6		
1.3	37.4	36.6	2.8
1.3	38.9		
1.3	33.4		
1.5	45.4	47.6	3.6
1.5	45.7		
1.5	51.8		
2.0	58.0	57.6	1.0
2.0	56.5		
2.0	58.3		
3.0	58.3	59.4	1.9
3.0	58.3		
3.0	61.6		

Table 4.4 Volumetric oxygen mass transfer coefficient values values for varying cultivation time in bubble column fungal fermentation; aeration rate of 1.5 vvm

Biomass Dry Weight (g)	Mean (h^{-1})	Std. Dev. (h^{-1})	N
0.00	47.6	3.6	3
4.93	49.4	2.3	3
5.77	48.1	3.0	3
5.82	56.6	4.0	4
6.53	62.2	0.8	4
7.39	40.9	2.6	3
7.89	51.9	2.9	3

4.3 Water-only Airlift and Bubble Column Volumetric Oxygen Mass Transfer Coefficient

Water-only ALR and BLR $k_L a$ values are presented in Tables 4.1 and 4.2. A scatter plot of $k_L a$ values versus aeration rate for airlift and bubble column configurations using water is shown in Figure 4.5. The plot shows that for 0.5 to 2.0 vvm, airlift and bubble column configurations share similar $k_L a$ values. A t-test performed for each aeration rate confirmed no statistical difference ($p \leq 0.05$) between the two configurations at each aeration rate, excluding 3.0 vvm.

The range for $k_L a$ values in ALR configuration was 17.1 ± 1.5 h^{-1} for 0.5 vvm to 100.8 ± 4.2 h^{-1} for 3.0 vvm. The range for $k_L a$ values in BLR configuration was 18.0 ± 0.6 h^{-1} for 0.5 vvm to 79.4 ± 2.2 h^{-1} for 3.0 vvm. Figures 4.6 and 4.7 shows a power regression between aeration rates 0.5 to 2.0 vvm for both configurations, with the best-fit equations being:

$$k_L a_{ALR} = 37.9 * vvm^{0.949}; R^2 = 0.9748$$

$$k_L a_{BLR} = 39.0 * vvm^{0.941}; R^2 = 0.9754$$

These power regressions correspond to the model, Equation 16, with $c = 37.9$ and $a = 0.949$ for ALR and $c = 39.0$ and $a = 0.941$ for BLR. Both exponents are less than 1, comparable to Deckwer et al. (1983) who reported values for *a* between 0.72 and 1.28 for saline solutions in a bubble column. The different units of v_s and *vvm* will cause the proportionality constant *c* to be dissimilar to Deckwer et al., however the two variables can be interchanged based on knowledge of the volume and cross sectional area of the bioreactor: $v_s = vvm * V/A_{Cross}$.

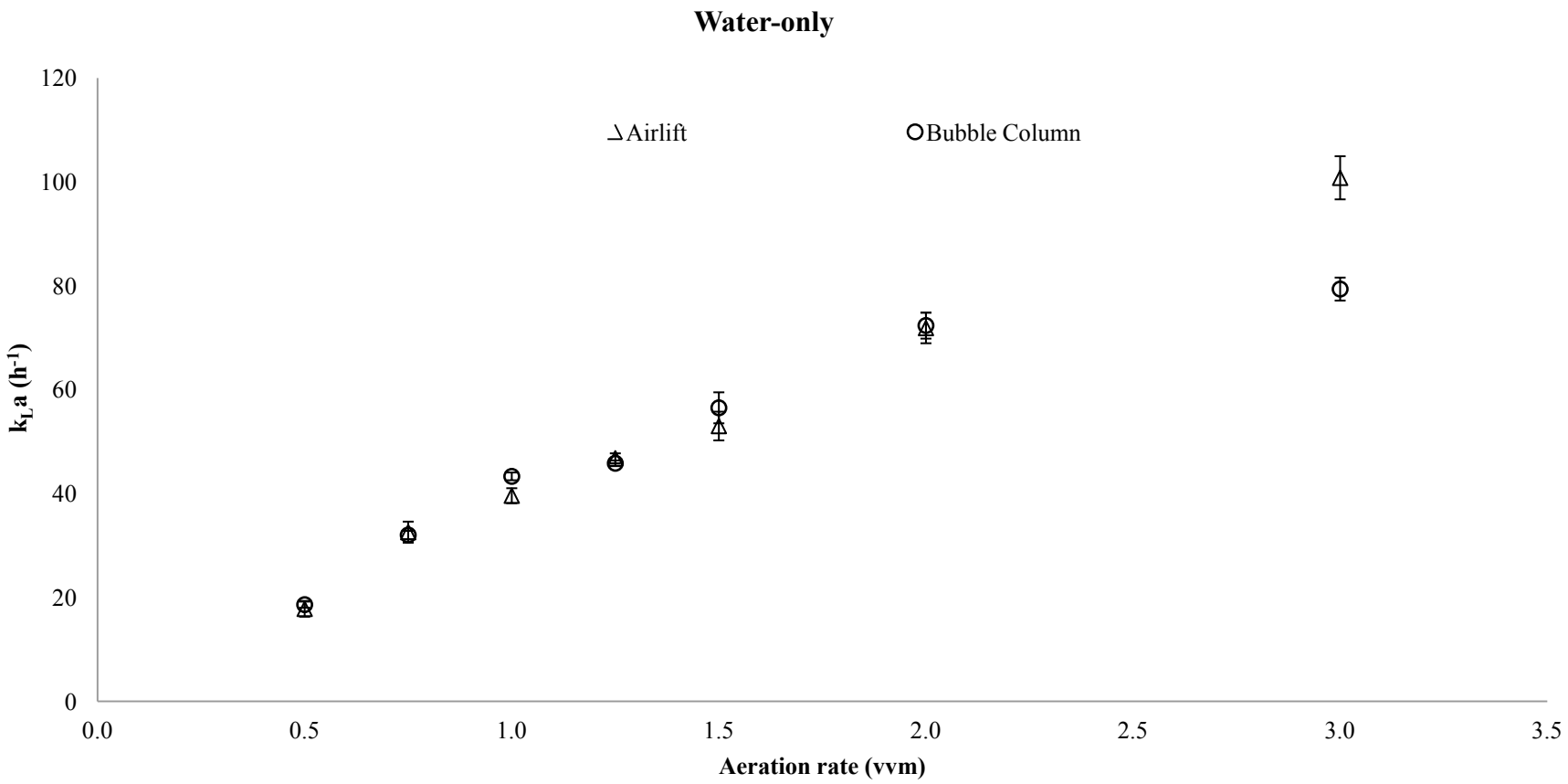


Figure 4.5 Water-only volumetric mass transfer coefficients for aeration rates 0.5- 3.0 vvm. n≥3. Vertical error bars represent 1 standard deviation from the mean

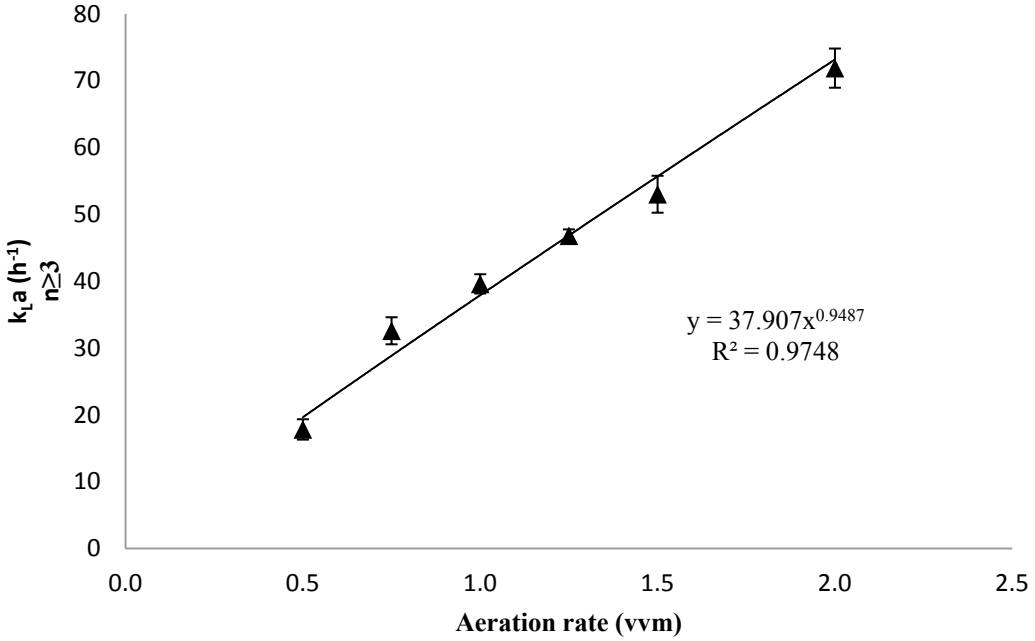


Figure 4.6 Power regression for water-only, airlift reactor, volumetric oxygen mass transfer coefficient values at aeration rates 0.5 to 2.0 vvm. Vertical error bars represent 1 standard deviation from the mean

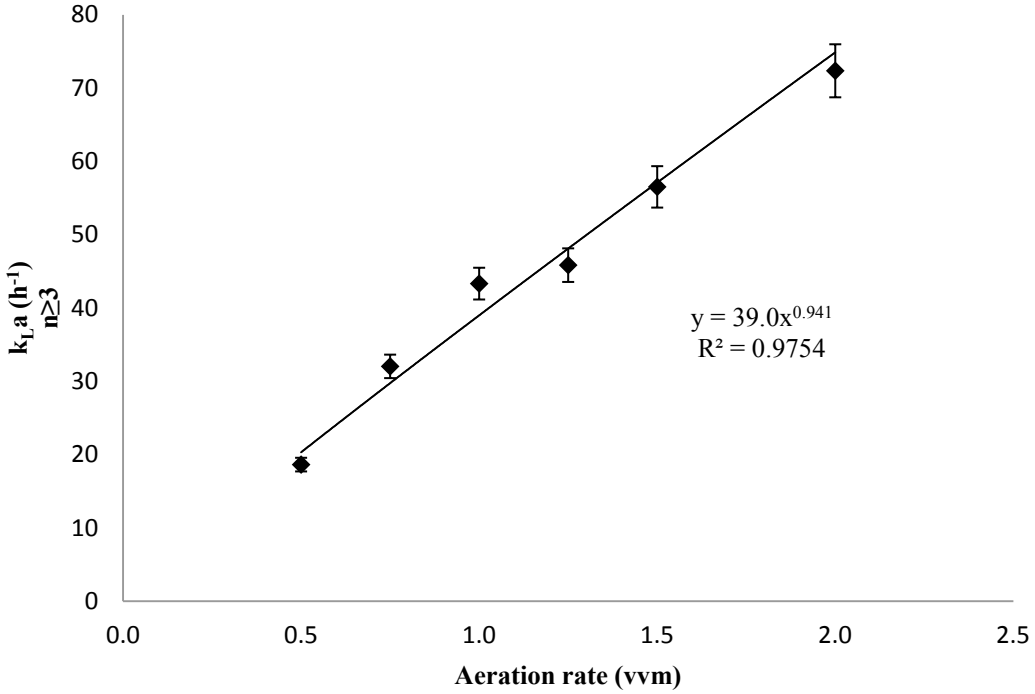


Figure 4.7 Power regression for water-only, bubble column reactor, volumetric oxygen mass transfer coefficient values at aeration rates 0.5 to 2.0 vvm. Vertical error bars represent 1 standard deviation from the mean

4.4 Vinasse-only Bubble Column Reactor Volumetric Oxygen Mass Transfer Coefficient

Vinasse-only BLR $k_L a$ values are presented in Table 4.3. A maximum $k_L a$ of 59.4 ± 1.9 h^{-1} was achieved at 3.0 vvm. All vinasse-only BLR $k_L a$ values were statistically lower ($p \leq 0.01$) when compared to water-only BLR $k_L a$ value, with a mean difference of 34.1 ± 17.7 % lower than the water-only value. Results from statistical analyses can be found in Table A.1.

Figure 4.8 shows a scatter plot of $k_L a$ values versus aeration rates for BLR configurations using vinasse-only. The S-curve type pattern suggests that at lower aeration rates, 0.5 to 1.25 vvm, $k_L a$ is suppressed and that as aeration increases greater than 1.25 vvm $k_L a$ tapers off to a maximum. The cause of lower $k_L a$ at lower aerations could be due to a multitude of factors including lower bubble rise velocity which increases the stagnant boundary layer, less bubbles which reduces the mixing force and turbulence which reduces gas hold up.

There were no experiments which measured the $k_L a$ values for aeration rates greater than 1.5 vvm because of the detrimental effects shearing had on fungal biomass morphology/yield (Nitayavardhana et al., 2013). However, the optimization experiments of Nitayavardhana et al. (2013) only studied 1.5 and 2.0 vvm. Possible future experiments could investigate if there is a more optimal aeration rate above 1.5 vvm and below 2.0 vvm, such as 1.75 vvm. This being said, understanding that increasing aeration will not increase $k_L a$ proportionally is important. While increased aeration increases power consumption (Blanch and Clark, 1997), there is also a limit to increasing $k_L a$ simply by increasing the aeration rate. This knowledge directly

impact costs consideration for commercial processes looking to increase oxygen mass transfer by increasing aeration rate (Mueller et al., 2002).

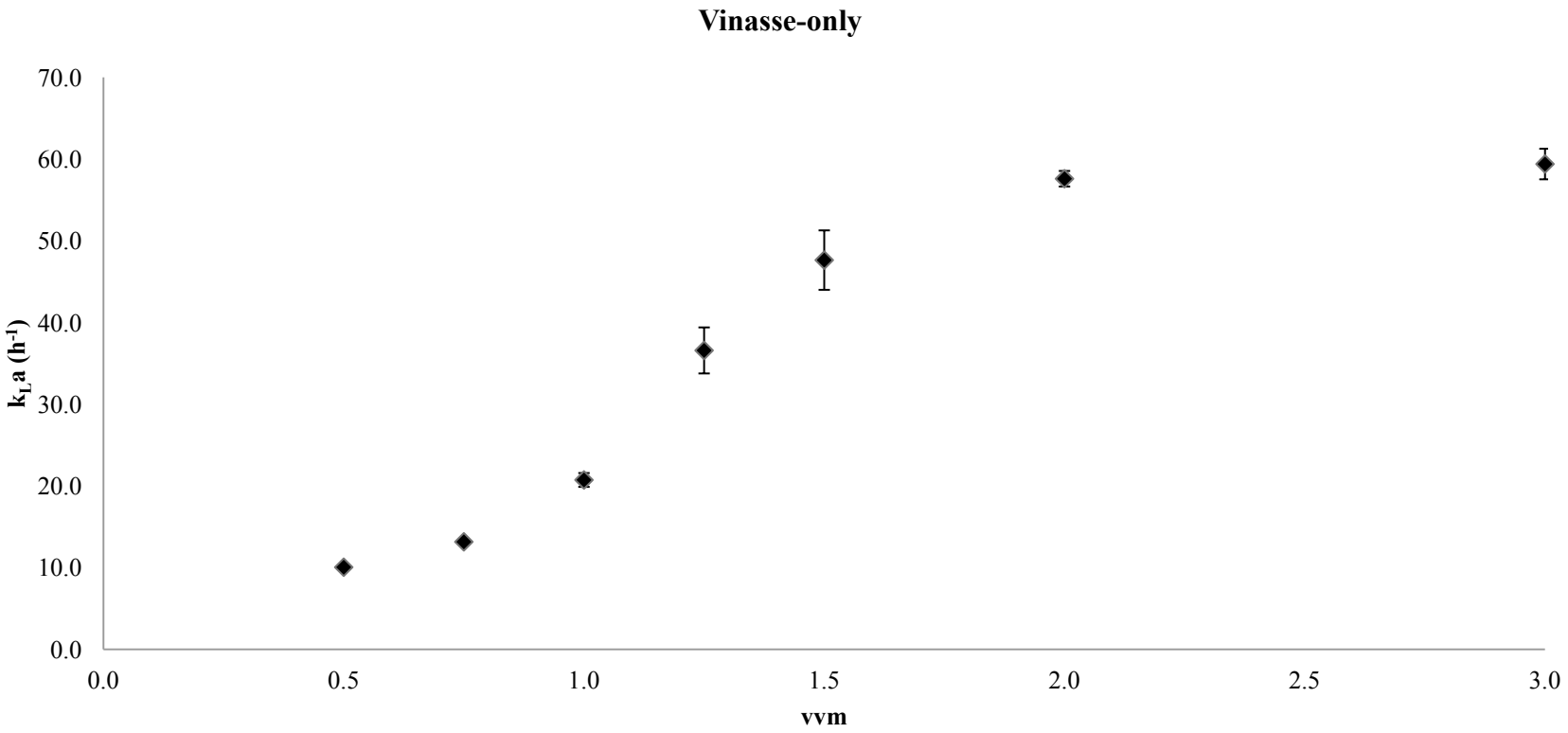
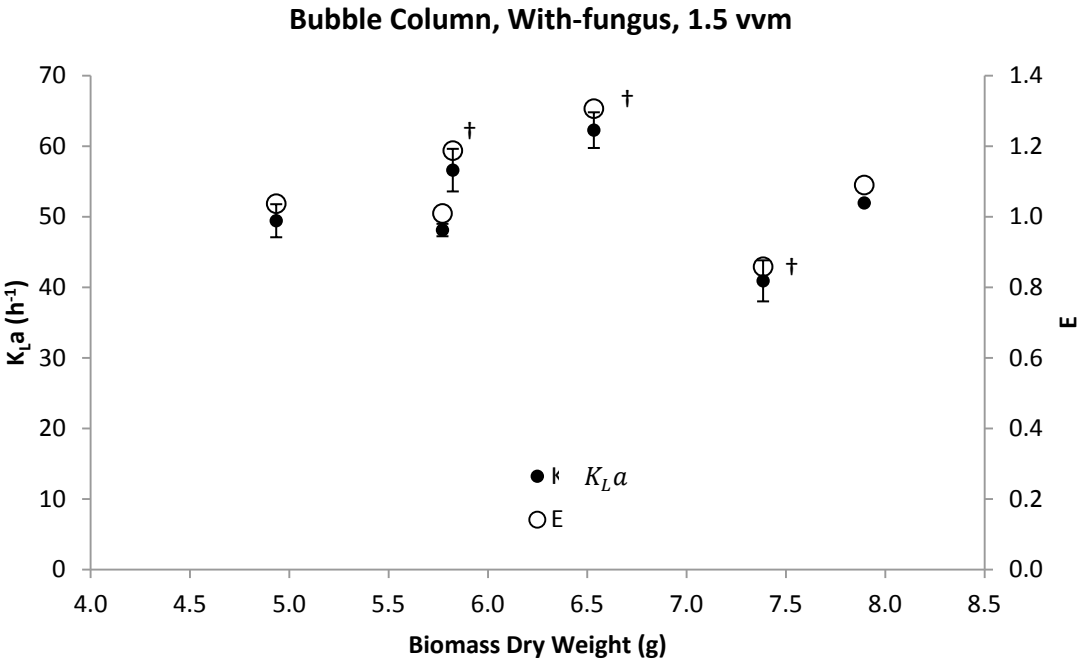


Figure 4.8 Vinasse only oxygen mass transfer coefficients for bubble column n=3. Vertical error bars represent 1 standard deviation from the mean.

4.5 With-fungus Volumetric Oxygen Mass Transfer Coefficient for Aeration Rate 1.5 vvm

$K_L a$ is presented as a function of biomass dry weight to illustrate the effect of fungal growth on oxygen mass transfer characteristics (i.e. biological enhancement factor). Figure 4.9 is a plot of $K_L a$ versus fungal biomass (g) dry weight and the enhancement factor, E (Equation 10), for each dry weight. Where, $k_L a = 47.64\ \mathrm{h}^{-1}$ for vinasse-only at 1.5 vvm, determined from Section 4.3.



†Values statistically different from water-only, 1.5 vvm.

Figure 4.9 With-fungus volumetric oxygen mass transfer coefficient (solid circles) for varying biomass dry weights (g) on the left hand axis. Enhancement factor (open circles) is on the right hand axis. Conditions: bubble column experiment with-fungus at 1.5 vvm. $K_L a_0$ is 47.64 h-1. Vertical error bars represent 1 standard deviation from the mean

Three biomass dry weights produced significant differences ($p \leq 0.02$) when comparing the $K_L a$ to $k_L a$ in BLR configurations at 1.5 vvm. When compared to vinasse-only $k_L a$, biomass dry weights 7.39 g had 14.1% reduction, 6.53 g a 30.7% increase, and

5.82 g and 18.8% increase at 1.5 vvm. It is important to highlight that for 6.53 g, the highest $K_L a$ measured at 62.3 h^{-1} is also significantly different from the mean water-only 1.5 vvm value, 55.2 h^{-1}. Table A.2 presents the statistical analysis for these claims.

When interpreting these results it is important to take into consideration the oxygen uptake rate of the fungus and yeast in the bioreactor. Higher biomass concentration and/or high specific oxygen uptake rate can increase the oxygen uptake rate. Both of these factors increase $K_L a$ according to Eqn. 11. This suggests that for 5.82 g and 6.53 g there was biological enhancement by either fungal biomass or yeast contamination. In Figure 4.9 the first five $K_L a$ values, which include 5.82 g and 6.53 g, correspond to growth times between 12-24 hours. This suggests that the fungus (and/or yeast) had significant specific oxygen uptake rate to increases $k_L a$because compared to higher biomass concentrations, 7.89 and 7.39 g, there was no significant biological enhancement over $k_L a$.

There was significant reduction (-14.1%) in oxygen mass transfer at the 7.39 g and 48 h of cultivation. This however does not rule out that $k_L a$ was increased by microbe OUR. It does however, mean that other factors reduced more than it was enhanced by microbe OUR. $K_L a$ is also dependent on other transient factors such as broth viscosity, bubble coalescence, bubble size, among others. This means that for instances where there is none or reduced biological enhancement, there are other reducing forces acting in tandem with microbe OUR. Observations during bioreactor operation such as increase bubble coalescence from the converging streamtubes caused by fungal biomass is present in the medium or increased broth viscosity from fungal and yeast cell biomass are possible causes of reduced $k_L a$.

Physical blocking effects by the fungal and yeast cells likely cause the reduction in $K_L a$ for fungal fermenation. For the fungal biomass dry weights 7.89 and 7.39 g, the fungus was harvested at or near the maximum biomass dry weight in this batch experiment—harvested at or after 48 h of cultivation (Nitayavardhana et al., 2013). If growth is limited at this time, this could mark a lower specific oxygen uptake rate which would correspond to lower biological enhancement. In addition, observations suggest that higher fungal biomass concentrations lead to increased bubble coalescence, increased contact with fungal pellets, and decreased physical contact with the bulk liquid. Increased apparent viscosity could have also lowered the $K_L a$. More studies are needed to differentiate between physical blocking and OUR consequences on $k_L a$ variance in fungal fermentation.

It was not possible to do $K_L a$ calculations past 48 hours of fermentation because oxygen uptake rate (Fungus and Yeast) would be greater than oxygen mass transfer leading to negative oxygen flux (Eqn. 5). When the oxygen flux is negative, the dynamic method is incapable of measuring $K_L a$. Pure oxygen was unavailable for this research, future experiments might consider using pure oxygen supplementation to investigate $K_L a$ when oxygen uptake rate outpaces oxygen mass transfer.

4.5 Observations

4.5.1 Airlift Reactor and Sparger Fouling

Bioreactor fowling during ALR operation caused results to be unreliable. During ALR operation, fungal biomass clumps would lodge in the space between the inner

reactor wall and draft tube. Fungal biomass also grew on and around the air diffusers as shown in Figure 4.10.

Figure 4.10 Air diffuser fouling in airlift reactor

DO probe fouling occurred when either bubbles or fungal biomass adheres to the oxygen membrane. This leads to either erratic DO readings or false $K_L a$ values. Possible solutions to this problem is to orient the DO probe membrane away from the liquid stream direction so the membrane does not create a stagnation point for bubbles or fungal clumps. A mesh could also be used to cover the membrane and prevent particulates from clogging the liquid/membrane interface.

4.5.2 Foaming

Foaming occurred at all aeration rates utilizing vinasse as a substrate (i.e. vinasse-only and fungal fermentations). If aeration rates were greater than 1.0 vvm at the beginning of fermentation, foaming would be excessive—filling the foam trap, entrapping the fungal pellets in the foam layer, and depositing pellets to the sidewalls, cap, and foam trap. Foaming could be controlled by keeping the aeration rate low during the beginning of the fungal cultivation (t<12-24 h). A chemical analysis of the vinasse before and after aeration should be done to verify what molecules are present before and after sterilization to confirm the claims by Junker (2007). After 12-24 hours of lower aeration, the aeration was increased to 1.5 vvm and the bioreactor had a stable foam height.

Junker (2007) notes that foam can be increased in the presence of cells due to increasing foam stability. In order to rule out the yeast cells as the cause for excessive foaming, yeast cells were removed by sedimentation. Even after yeast cell removal, the supernatant vinasse would still foam excessively at high aeration rates. This suggests that it is the soluble components of vinasse, sugars and ethanol left over from ethanol fermentation, cause excessive foaming in vinasse. Junker (2007) noted that 1-22% glucose concentrations stabilize foam and that sugar containing medias foam excessively when sterilized with high temperature treatment. Van't Reit and Tramper (1991) reported that foaminess is a maximum at 1-2% (v/v) short chain alcohols.

CHAPTER 5 CONCLUSIONS

This research investigated the volumetric oxygen mass transfer coefficient, $k_L a$ and $K_L a$, in abiotic and fungal fermentation. The fungus grew in a bubble column bioreactor with vinasse as the substrate. Nitayavardhana et al. (2013) reported 1.5 vvm being the optimal aeration rate and this research measured an oxygen mass transfer coefficient for the same aeration rate, with results ranging from 40.9 ± 2.6 h^{-1} to 62.2 ± 0.8 h^{-1}. These values are in the comparable to previous BLR experiments within the same superficial gas velocity (Vandu and Krishna, 2004). Conditions were identical to Nitayavardhana et al. (2013) with the exception that there was no nutrient supplementation in this research. The deciding factor for whether nutrient supplementation should be used is an economic analysis, which should be done in the future.

In an effort to be familiar with how aeration rate affects $k_L a$ in the bioreactor, the research measured water-only and vinasse-only $k_L a$ values for varying aeration rates in the range of 0.5 to 3.0 vvm. We obtained empirical equations for ALR and BLR configurations using water-only media:

$$k_L a_{ALR} = 37.9 * vvm^{0.949}\,; R^2 = 0.9748$$

$$k_L a_{BLR} = 39.0 * vvm^{0.941}\,; R^2 = 0.9754$$

These equations are a guide to determining the range of $k_L a$ values for any aeration rate within the range of aeration rates for this experiment (0.5 to 2.0 vvm). Future experiments should test the robustness at aeration rates between this experiment's measurements (i.e. 1.33 vvm) to validate the model's robustness. While the model is

based on using superficial gas velocity a successfully scaled-up BLR experiment by Deckwer et al. (1974), the geometry of the BLR does not allow these specific equations based on vvm to be used during scale-up. However, using the method from this research to determine a relationship based on aeration rate should be done before the actual fungal fermentation to give a range of $k_L a$ values at each aeration rate.

Vinasse-only experiments did not show power curves at lower aeration rates. On the other hand, we observed a logistical growth curve—exponential increasing at first then approaching a limiting value. The minimum $k_L a$ was 10.1 ± 0.2 h^{-1} at 0.5 vvm and maximum $k_L a$ of 59.4 ± 1.9 h^{-1} at 3.0 vvm for the BLR configuration. When comparing water-only to vinasse-only aeration rates for BLR configuration, it was shown that $k_L a$ for vinasse media was on average 34.1± 17.7 % lower than water. The reduction in oxygen mass transfer is very significant, as good dissolved oxygen levels are crucial for promoting high biomass yields.

This research did not investigate the exact cause of the decreases in $k_L a$. It was not feasible to conduct enough controlled studies to determine the independent causes of reduced $k_L a$ values, such as changes in bubble size, viscosity, and gas hold-up. More research is needed to determine the underlying causes that reduce $k_L a$. Through observations, we suspected that low oxygen mass transfer was a limiting factor to fungal growth. This researched did determine that the presence of fungal biomass (and yeast cells) can enhance or reduce $K_L a$ at certain stages of growth. If the fungal biomass is high in concentration and specific oxygen uptake rate, $K_L a$ will be enhanced in this situation. However, if the fungal biomass is in a declining phase of growth, and there is a lower OUR, the factors that reduce $K_L a$ will outweigh the increases due to OUR (Kilonzo and

Margaritis, 2004). Yeast contamination may also enhance $K_L a$, however a sterilization study needs to be done to confirm overall effects of yeast cells on $K_L a$.

We did not investigate the ALR configuration for vinasse media because we observed reactor fouling and fungal pellet clumping in this configuration. Chapter 6 discusses possible solutions and future research with airlift reactors.

The objective to obtaining a standard curve of $k_L a$ values for varying aeration rates through empirical methods was successful. This is the first step in planning a commercial fungal fermentation unit process. Armed with this knowledge of oxygen mass transfer in fungal fermentation, engineers and future researchers can possibly tailor aeration rates to match the fungal oxygen uptake rate. This will allow engineers to set optimal growth conditions of minimizing shear and maintaining sufficient DO levels resulting in the most efficient bioreactor operation. Finally, by reducing the aeration costs for fungal fermentation, it will further increase the chances for the unit process to replace the traditional method of disposing vinasse directly into the environment.

CHAPTER 6 FUTURE RESEARCH AND DIRECTIONS

This research investigated oxygen mass transfer in airlift and bubble column bioreactors growing a mycelia fungus on a complex liquid biofuel residue, vinasse. It also focused on the effects of aeration on the volumetric oxygen mass transfer coefficients, $k_L a$ and $K_L a$. However, there are variables other than aeration that influence $k_L a$ and $K_L a$ values. Future research should include:

1. The effects of transient changes in broth viscosity on $K_L a$. Broth viscosity can be reduced by the uptake and utilization of sugars in vinasse during fungal fermentation. This would lead to increased $K_L a$. However, reduced sugar levels correspond to increased fungal biomass concentrations and yeast concentrations, which are likely to increase broth viscosity. An investigation into effluent vinasse compared to influent vinasse broth viscosity should be done.
2. The effects of bubble sizes on fungal yields and other bioreactor performance measures. Bubble size has a dramatic effect on oxygen mass transfer as well as other fermentation conditions such as bubble rise velocity, shear rates, and bulk mixing. Effects for different spargers and aeration rates should be investigated for effects on bioreactor performance and fungal biomass yield.
3. An investigation to fungal cultivation in a larger ALR. The ALR can improve mixing and increase gas hold-up. However due to fungal growth, the oxygen mass transfer could not be investigated for the 2.5 L ALR used in this experiment. Future experiments should scale up and include larger ALR designs, which can have larger spaces in downcomer and riser sections. Fungal pellets have limited maximal size, while the downcomer and riser sections will increase with scale-up,

which should prevent fouling and allow for $K_L a$ measurements. DO probe placement with the membrane pointing in the same direction as the liquid flow (i.e. right side up in the downcomer or upside down in the riser) will prevent probe fouling by preventing fungal attachment and bubble entrapment.

4. Currently no method is available to characterize the cell concentration filamentous fungi. This makes prediction of the biological enhancement factor nearly impossible. A method needs to be developed that measures the specific oxygen uptake rate based on fermentation time
5. Use of other methods to improve $K_L a$. $K_L a$ can be improved by using mechanically agitating ALRs and BLRs or using hollow-fiber membranes for oxygen mass transfer. Nevertheless, precautions to prevent morphology changes and fouling should be planned before entering into these advanced methods of oxygen mass transfer.
6. An economic analysis of aeration costs should be investigated to determine if scale-up of fungal fermentation is feasible. Larger scale data is also required for this analysis.
7. A costs-benefit analysis of nutrient supplementation should also be investigated to determine whether it is appropriate to supplement nutrients. Whether or not to supplement nutrients may affect fungal protein profiles along with macromolecule composition, which impacts the feed value/potential of the fungal biomass
8. Regarding vinasse-only operation (Section 3.2.6), 500 mL of YM broth would be a better control than 500 mL of water due to solutes present in YM broth for with-fungus experiments.

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APPENDIX

A.1 Data Anaylsis

Table A.1 Comparison of water-only to vinasse-only volumetric oxygen mass transfer coefficients in bubble column: t-test assuming equal variances

0.5 vvm		
	Water-Only	*Vinasse-Only*
Mean	18.6	10.1
Variance	0.3	0.0
Observations	3	3
Pooled Variance	0.2	
Hypothesized Mean Difference	0	
df	4	
t Stat	24.39	
P(T<=t) one-tail	8.38E-06	
t Critical one-tail	2.13	
P(T<=t) two-tail	1.68E-05	
t Critical two-tail	2.77	
0.75 vvm		
	Water-Only	*Vinasse-Only*
Mean	32.0	13.2
Variance	0.6	0.1
Observations	3	3
Pooled Variance	0.4	
Hypothesized Mean Difference	0	
df	4	
t Stat	37.79	
P(T<=t) one-tail	1.46E-06	
t Critical one-tail	2.13	

P(T<=t) two-tail	2.93E-06	
t Critical two-tail	2.78	
1.0 vvm		
	Water-Only	*Vinasse-Only*
Mean	43.3	20.7
Variance	0.6	0.7
Observations	3	3
Pooled Variance	0.6	
Hypothesized Mean Difference	0	
df	4	
t Stat	34.60	
P(T<=t) one-tail	2.08E-06	
t Critical one-tail	2.13	
P(T<=t) two-tail	4.16E-06	
t Critical two-tail	2.78	
1.25 vvm		
	Water-Only	*Vinasse-Only*
Mean	45.8	36.6
Variance	0.3	7.9
Observations	3	3
Pooled Variance	4.1	
Hypothesized Mean Difference	0	
df	4	
t Stat	5.58	
P(T<=t) one-tail	0.0025	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.0050	
t Critical two-tail	2.77	
1.5 vvm		

t-Test: Two-Sample Assuming Equal Variances		
	Water-Only	*Vinasse-Only*
Mean	56.5	47.6
Variance	8.9	13.3
Observations	4	3
Pooled Variance	10.6	
Hypothesized Mean Difference	0	
df	5	
t Stat	3.56	
P(T<=t) one-tail	0.0081	
t Critical one-tail	2.02	
P(T<=t) two-tail	0.0162	
t Critical two-tail	2.57	
2.0 vvm		
	Water-Only	*Vinasse-Only*
Mean	72.4	57.6
Variance	6.4	0.9
Observations	3	3
Pooled Variance	3.6	
Hypothesized Mean Difference	0	
df	4	
t Stat	9.49	
P(T<=t) one-tail	0.0003	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.0006	
t Critical two-tail	2.78	
3.0 vvm		
	Water-Only	*Vinasse-Only*
Mean	79.4	59.4

Variance	4.9	3.5
Observations	4	3
Pooled Variance	4.3	
Hypothesized Mean Difference	0	
df	5	
t Stat	12.5	
P(T<=t) one-tail	3E-05	
t Critical one-tail	2.01	
P(T<=t) two-tail	6E-05	
t Critical two-tail	2.57	

Table A.2 Comparison of with-fungus volumetric oxygen mass transfer coefficient to vinasse-only oxygen mass transfer coefficient in bubble column reactor: t-test: two-sample assuming equal variances

	Vinasse-Only	*4.93 g*
Mean	47.6	49.4
Variance	13.3	5.5
Observations	3	3
Pooled Variance	9.4	
Hypothesized Mean Difference	0	
df	4	
t Stat	-0.720	
P(T<=t) one-tail	0.255	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.511	
t Critical two-tail	2.77	
	Vinasse-Only	*7.89 g*
Mean	47.6	52.0
Variance	13.3	8.5
Observations	3	3

Pooled Variance	10.9	
Hypothesized Mean Difference	0	
df	4	
t Stat	-1.60	
P(T<=t) one-tail	0.0919	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.184	
t Critical two-tail	2.778	
	Vinasse-Only	*7.39 g*
Mean	47.6	40.9
Variance	13.3	6.4
Observations	3	3
Pooled Variance	9.8	
Hypothesized Mean Difference	0	
df	4	
t Stat	2.62	
P(T<=t) one-tail	0.029	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.058	
t Critical two-tail	2.78	
	Vinasse-Only	*6.53 g*
Mean	47.6	62.3
Variance	13.3	0.8
Observations	3	4
Pooled Variance	5.8	
Hypothesized Mean Difference	0	
df	5	
t Stat	-7.97	
P(T<=t) one-tail	0.0002	

t Critical one-tail	2.02	
P(T<=t) two-tail	0.0004	
t Critical two-tail	2.57	
	Vinasse-Only	*5.77 g*
Mean	47.6	48.1
Variance	13.3	9.1
Observations	3	3
Pooled Variance	11.2	
Hypothesized Mean Difference	0	
df	4	
t Stat	-0.176	
P(T<=t) one-tail	0.435	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.869	
t Critical two-tail	2.78	
	Vinasse-Only	*5.82 g*
Mean	47.6	56.6
Variance	13.3	16.1
Observations	3	4
Pooled Variance	15.0	
Hypothesized Mean Difference	0	
df	5	
t Stat	-3.03	
P(T<=t) one-tail	0.014	
t Critical one-tail	2.01	
P(T<=t) two-tail	0.029	
t Critical two-tail	2.57	

A.2 The Current State of Ethanol Production

Currently, more than 20 billion gallons of bioethanol is produced annually worldwide. Ethanol is a primary replacement for transportation fuel with E10 (10% ethanol blends) in 96% of United States gasoline as of 2012. (Renewable Fuels Association, 2013) Figure A.1 shows the growth ethanol production since 2006.

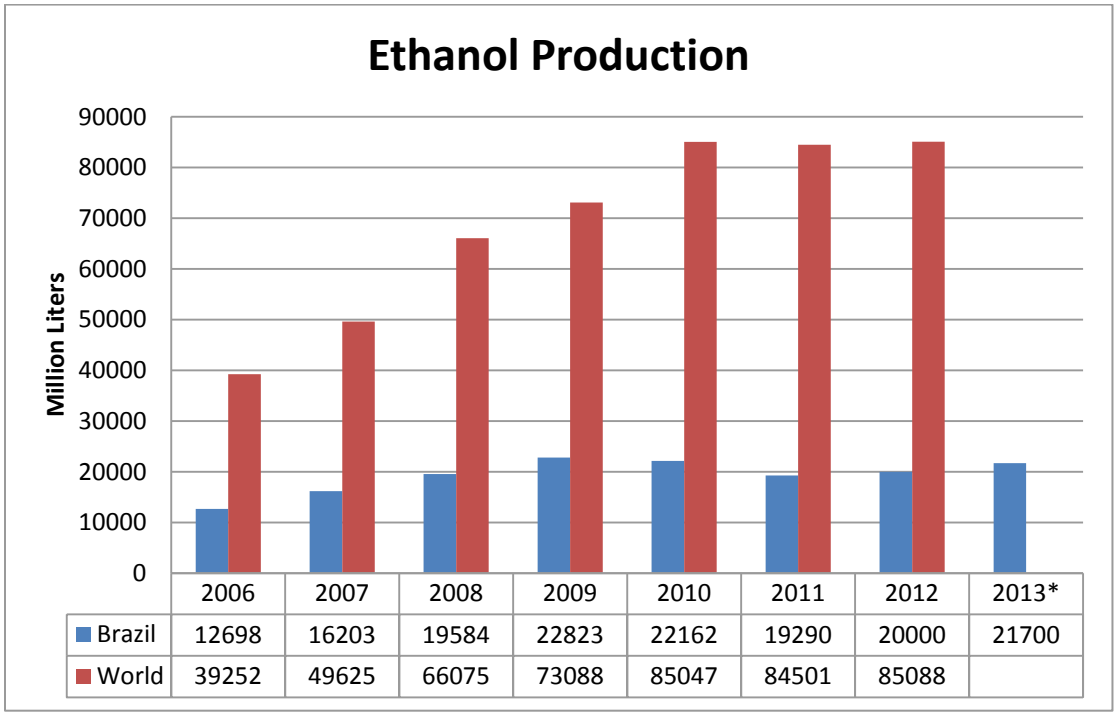


Figure A.1 Worldwide ethanol production from 2006 to 2012 (Renewable Fuels Association, 2013)

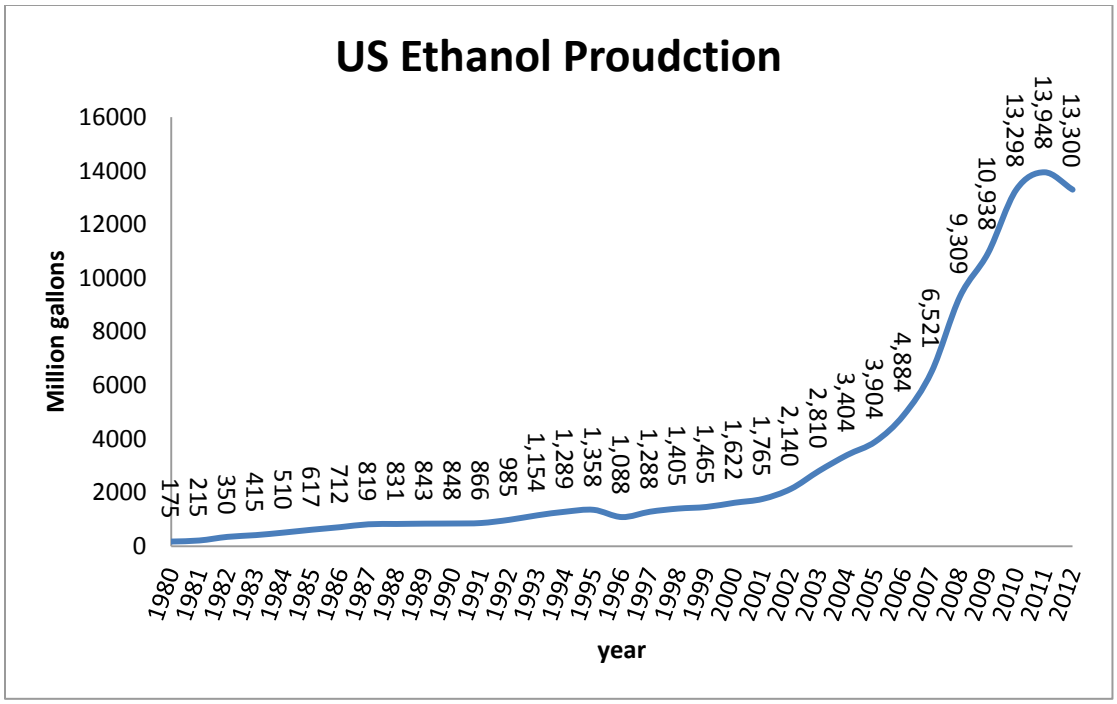


Figure A.2 US ethanol production from 1980 to 2012 (Renewable Fuels Association, 2013)

In an effort to gain energy independence the US government passed the Energy Independence and Security Act of 2007. In the legislation, the US is required to increase biofuels added to the gasoline supply to 36 billion gallons in year 2022 from 4.7 billion gallons in 2007. The legislation also specifies that 22 billion gallons of the biofuel must come from non-cornstarch products (i.e. sugar and cellulose) (P.L.110-140, H.R. 6).

The legislation lead to the exponential growth of US ethanol production from 1980 to 2010 (Figure A.2). However, the past three years, 2010-2012 has seen the production stagnate at nearly 20 billion barrels worldwide, and 14 billion barrels domestically. However, in a 2012 report, the US EIA noted that currently the US ethanol production has met all demand for E10 gasoline (i.e. 10% ethanol blended into petroleum gasoline). It is now, during a stagnation in ethanol production growth, that the US, and

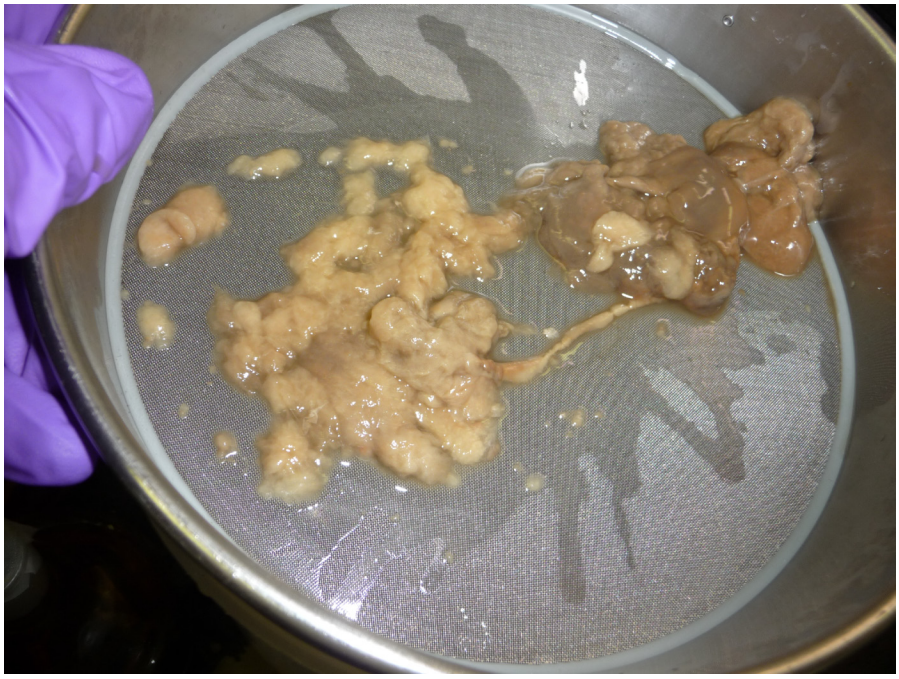
the world must develop new co-product technologies which will increase the value of the co-products generated.

A.3 Photos

Fungal plugging between draft tube and sidewall.

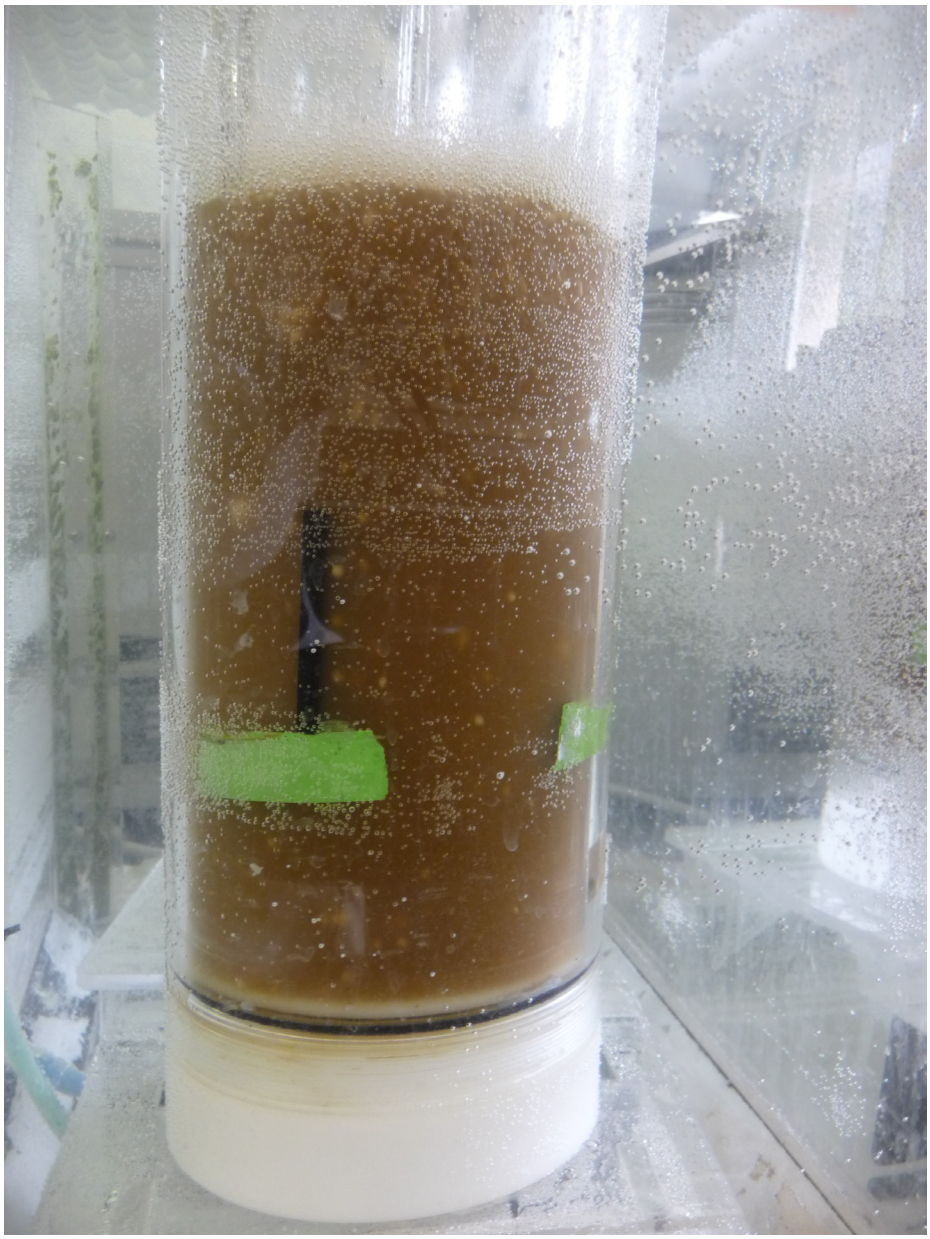
Rotovapor: For ethanol distillation

Hawaiian Commercial and Sugar Co., Maui Sugar Mill, molasses storage tanks in front and power plant in the background (2011)



Harvested Fungal pellets

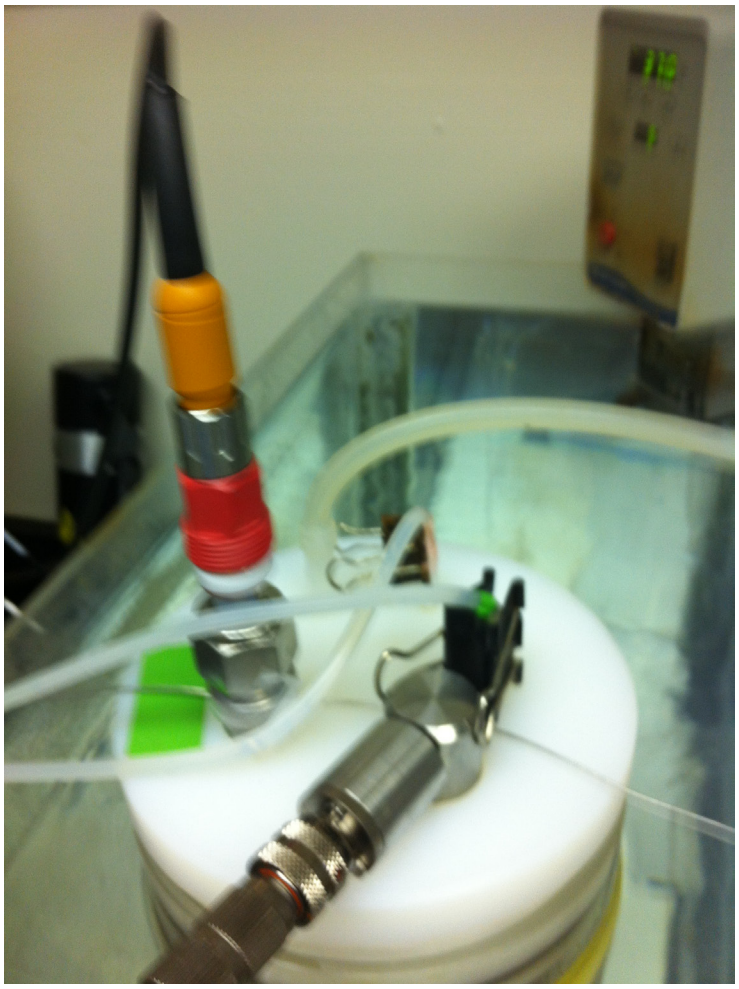
Fungal growth over and around the sparger



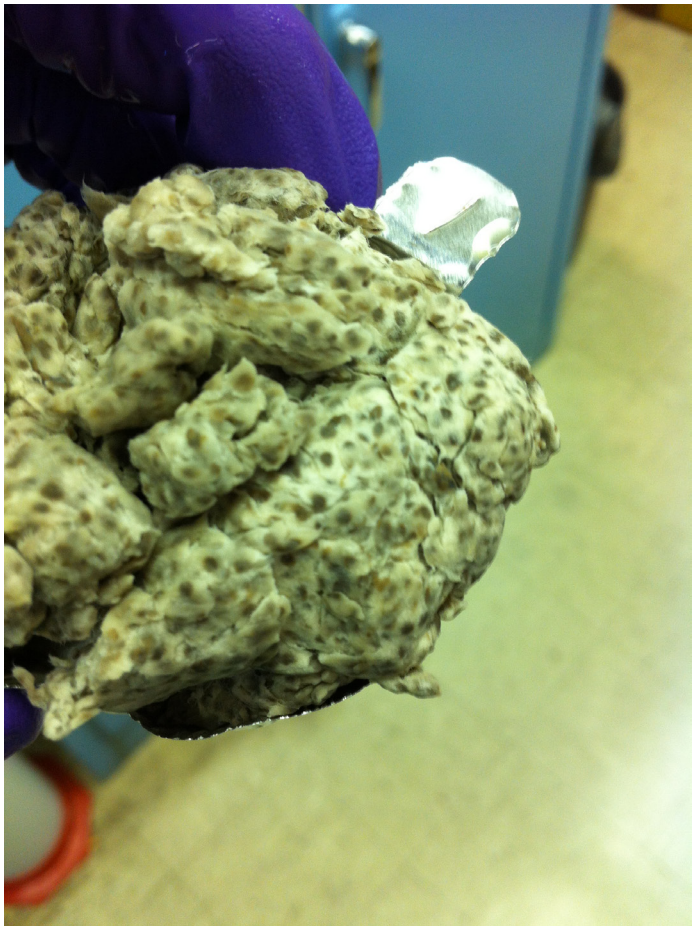
Fungal fermentation in airlift bioreactor in water bath, vinasse media

Freeze Dried Fungal Pellets

Prototype external loop airlift bioreactor, PVC pipe. Could not be heated, leaked, fungus probably would get stuck in the 1" downcomer tube.



Top of airlift bioreactor. pH probe (orange) and DO probe, lower right



Washed and compressed fungal pellets