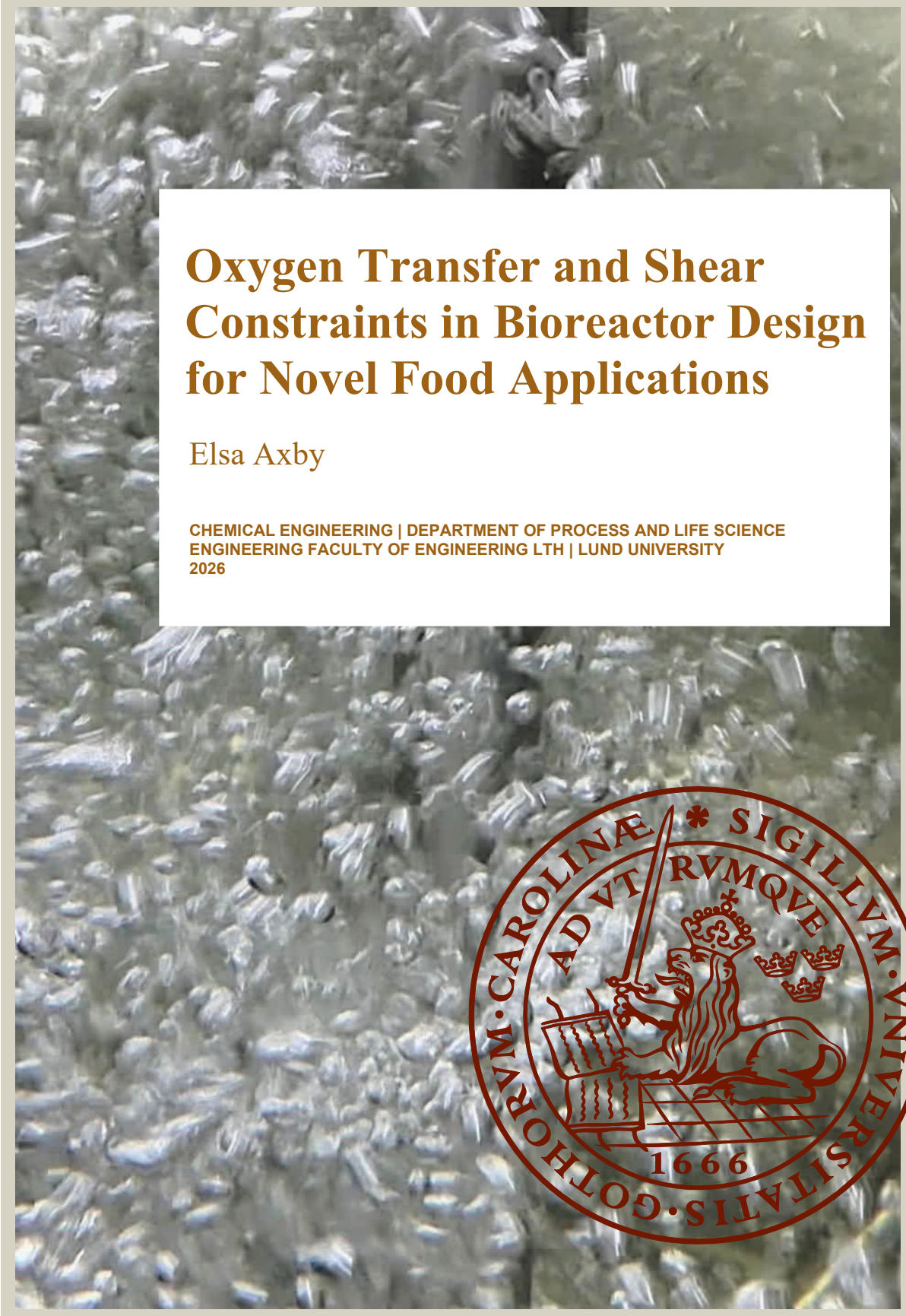




Oxygen Transfer and Shear Constraints in Bioreactor Design for Novel Food Applications

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CHEMICAL ENGINEERING | DEPARTMENT OF PROCESS AND LIFE SCIENCE
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2026



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Defining Operational Windows for a Modular Pilot-Scale Bioreactor

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by

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Process and Life Science Engineering

June 2026

Supervisor: Niklas Andersson
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Finding the Sweet Spot for the Food of the Future

Imagine if the milk in your coffee or the meat in your hamburger came from a fermenter instead of a methane producing cow. This thesis identifies specific operating conditions for growing animal cells and bacteria in bioreactors, making the production of sustainable food of the future possible.

How we produce food today has an enormous impact on our climate, and with a growing population, new ways to produce the food we rely on must be developed. By placing cells in fermenters and feeding them with sugars and other nutrients, they can grow into meat or key food ingredients. Cultivated meat can reduce greenhouse gas emissions by 92% compared to conventional meat, and alternative proteins can reduce water usage by up to 70%. Today, there is a lot of ongoing development of cell strains that can produce different ingredients, like alternative milk proteins or palm oil, that give the consumer the same experience as traditional products. However, the process is not only biology, but also an engineering challenge. The fermenters, in which the cells grow, must be more efficient so that prices of the products can compete with what is already on the market today. Similar fermenters have long been developed for the pharmaceutical industry, but to produce food, the financial margin is much smaller, meaning that the fermenter design and the process efficiency have to be higher to make it profitable.

To reach a high process efficiency, the environment in the fermenter should be as close to the cell's optimum as possible. One of the largest challenges in doing so is that it is difficult to give the cells enough oxygen without the air making the cells explode due to the forces. This balance is hard to find and is specific for each fermenter and cell type. Therefore, this thesis has found a "safe zone" where specific cells are growing with enough oxygen, while the environment is gentle enough to make them thrive. This was done for a robust bacteria, *Escherichia coli*, and for a more delicate animal cell type, CHO cells.

Four different "bubbling methods" and different gas flows were tested to find how fast oxygen dissolves in liquid under certain conditions. Then, how much stress this bubbling created for the cells was calculated. Using this information, a sweet spot for both *E. coli* and CHO cells was found. *E. coli* needs a lot of oxygen, meaning that the bubbling method that is the most efficient was the best. CHO cells, on the other hand, are so fragile that even the popping of a bubble can kill them, meaning that only more gentle bubbling methods could be used.

Since growing cells in fermenters is still quite expensive, the conditions in which it is done should be well selected. Using the sweet spot found in this thesis as a guideline, it will be easier to find the best method to give the cells the oxygen they need, without killing them while doing so. This is a step towards producing sustainable food at a larger scale, while not changing the consumer experience.

Abstract

Current agricultural practices are not sustainable to meet the demands of a growing global population, requiring alternative methods to produce food ingredients, such as proteins, oils, and fats. The emerging field of novel food, including precision fermentation and cultivated meat, presents new approaches, but needs further research to reach scalable commercial viability. Improved understanding of precision fermentation and cultivated meat is needed to optimize the process conditions, particularly regarding oxygen transfer and shear forces to support efficient cell cultivation. However, the interdependency between these two parameters is complex and must be defined for each reactor setup and operating condition. This thesis aims to identify an operational window regarding aeration and shear forces, with consideration for both oxygen requirements and shear stress constraints for two representative and well documented cell cultures for precision fermentation and cultivated meat, being *Escherichia coli* and CHO cells.

With a 310-liter bioreactor as a pilot scale representative for operating conditions, four aeration methods, sintered stone, pipe on pipe aeration with and without agitation, and venturi valve, were evaluated at different gas flows. Using the dynamic method, the volumetric mass transfer coefficient was found for each operating condition. The results were compared to the oxygen requirements of *E. coli* and CHO cells to find the minimum gas flow needed for each of the aeration methods. The shear forces created by agitation, both locally and the average shear, and shear by aeration, both by the sparging and the gas bubbles rupturing, were calculated and compared to lethal levels for *E. coli* and CHO cells.

The results identified an operational window for each aeration method. The oxygen requirements defined the lower limit, and shear forces set the upper limit on gas flow. The gas bubbles rupturing was found to eliminate some aeration configurations. The local shear forces by agitation defined an upper limit for agitation.

With the operational windows for each aeration method together with the found oxygen transfer efficiency, a recommendation on aeration conditions is proposed for the used bioreactor setup. For *E. coli*, aeration with the venturi valve at a gas flow rate of 18 m³/h is recommended. For CHO cells, pipe on pipe aeration with a maximum agitation of 1.2 /s with a gas flow rate of 0.8 m³/h is recommended. However, validation of the results must be made to confirm the accuracy and the scalability of operations for industrial scale.

Sammanfattning

Dagens jordbruk är inte hållbart för att möta behoven hos en växande befolkning. Detta kräver alternativa metoder för att producera livsmedelsingredienser som proteiner, oljor och fett. Det framväxande området av nya livsmedel, med precisionsfermentering och odlat kött, utgör nya möjligheter, men det behöver ytterligare forskning för att uppnå kommersiell lönsamhet. Det krävs bättre förståelse av precisionsfermentering och odlat kött för att optimera processparametrar, speciellt gällande syreöverföring och skjuvkraft för att möjliggöra effektiv cellodling. Sambandet mellan dessa två parametrar är dock komplext och behöver definieras för varje bioreaktor och driftförhållande. Syftet med denna uppsats är att identifiera driftintervaller för luftning och skjuvkrafter, med hänsyn till syrebehov och begränsningar i maximal skjuvning för två representativa cellkulturer för precisionsfermentering och odlat kött, *Escherichia coli* och CHO celler.

En 310 liter bioreaktor i pilotskala användes för att undersöka olika driftförhållanden. Fyra luftningsmetoder, sintrad sten, rör-mot-rör med och utan omrörning och venturiventil, utvärderades vid olika gasflöden. Den dynamiska metoden användes för att hitta den volymetriska massöverföringskoefficienten för varje driftförhållande. Resultaten jämfördes med syrebehoven hos *E. coli* och CHO celler för att hitta lägsta gasflödet för varje luftningsmetod. Lokala och genomsnittliga skjuvkrafter som skapas vid omrörning beräknades. Skjuvning från luftning, både vid luftinjektion och när gasbubblor spricker, beräknades och jämfördes med dödlignivå för *E. coli* och CHO celler.

Resultaten skapade ett driftsfönster för varje luftningsmetod. Cellernas syrebehov utgör den lägre gränsen, medan skjuvkrafterna utgör den högre gränsen för gasflödet. Sprickandet av bubblor fanns utesluta några driftskombinationer. Lokala skjuvkrafter från omrörning satte en övre gräns för skjuvkraft.

Med informationen från driftsfönstret för varje luftningsmetod och luftningens effektivitet kan driftförhållanden rekommenderas för den undersökta bioreaktorn. För *E. coli* rekommenderas att använda venturiventil med ett gasflöde av 18 m³/h. För CHO celler rekommenderas att använda rör-mot-rör metoden med omrörning upp till 1.2 /s och ett gasflöde på 0.8 m³/h. Fler försök krävs för att validera resultatens noggrannhet och möjligheten att skala upp processen till industriell skala.

Acknowledgement

I would like to thank Curve Biotech for letting me use their bioreactors and materials throughout this project. Being able to use the pilot scale equipment gave a lot of important experience and understanding of process operations and project development.

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1 Background

This thesis is based on work conducted with Curve Biotech. Curve Biotech is a start-up company developing a combined hardware and software system for scaling up biomanufacturing processes in precision fermentation and cell cultivation. Together with strain developing partners, Curve Biotech develops and scales up processes for the production of food ingredients, such as proteins, oils, fats, and enzymes. With increasing interest in scaling up mammalian cell cultivation, production of cell mass for cultivated meat is also of high interest.

When the research for this thesis was being conducted, Curve Biotech recently designed and produced a bioreactor setup for biomanufacturing, mainly in the novel food field. The hardware configuration consists of a rig with two bioreactors, with different designs, each with an operational volume of 310 l and a control unit. The setup has a unique modular design that can be adjusted to meet specific process requirements. As aeration is recognized as one of the main challenges in biomanufacturing, the setup has a flexible, interchangeable aeration system that can be combined with several types of agitation. Therefore, the aeration and agitation can differ depending on whether an oxygen requiring bacteria, or a shear sensitive mammalian cell is cultivated.

All experimental work presented in this thesis was conducted using bioreactors by Curve Biotech and was conducted solely by Elsa Axby.

In this thesis, artificial intelligence has been used to generate code for visualization of data.

2 Aim of the thesis

To improve the efficiency and economic viability of precision fermentation and cultivated meat processes, this thesis aims to provide operational guidelines for aeration for the cultivation of bacteria and mammalian cells in pilot scale reactors. In fermentation in bioreactors, aeration and shear forces are interdependent parameters, where increasing aeration will increase shear stress on cells. How the parameters vary is specific to each bioreactor configuration and must therefore be studied for each system.

Using a 310 liter bioreactor, four different aeration methods are compared and evaluated across a range of gas flows to determine the volumetric mass transfer coefficient (k_La) using the dynamic method. The gas flow required to meet the oxygen requirement for two representative cell cultures for precision fermentation and cultivated meat, *Escherichia coli* and Chinese hamster ovary (CHO) cells, is investigated. These are selected due to being well documented and for their importance in bioprocessing.

Further, the shear forces created by aeration will be estimated and compared to reported lethal levels for these cell cultures. This will include both shear forces from gas sparging and the rupture of gas bubbles. One of the aeration methods includes agitation for which both local and average shear forces are determined.

Finally, the aim is to define an operational window for each aeration method where the lower limit is defined by the oxygen requirements, and the upper limit is defined by the maximum shear forces. This provides a framework for further experimental trials and can guide when scaling up reactors for precision fermentation and cultivated meat to industrial size.

3 Nomenclature list

	Quantity	Unit
Oxygen concentration at saturation	$C_{O_2}^*$	mg/l
Oxygen concentration	C_{O_2}	mg/l
Diameter of bubble	d_b	m
Diameter of sparger hole	d_h	m
Diameter of impeller	D_i	m
Dry cell weight	dcw	g
Energy dissipation rate	EDR	W/m ³
Gas entrance velocity	GEV	m/s
Volumetric oxygen mass transfer coefficient	k_La	/min
Power number	N_p	-
Stirrer speed	N_i	/s
Number of impellers	n_i	-
Number of holes in sparger	n_h	-
Power input by agitator	P	W
Gas flow rate	Q_g	m ³ /h
Oxygen consumption	q_{O_2}	mmol/g dcw*h mmol/cell*h
Time	t	min
Volume of reactor	V	m ³
Cell density	x	g dcw /l cells/l
Liquid density	ρ	kg/m ³
Impeller tip speed	v_i	m/s

4 Theoretical background

4.1 Novel food

According to EFSA, a novel food is defined as “...food that had not been consumed to a significant degree by humans in the EU before 15 May 1997, when the first Regulation on novel food came into force.” EFSA further describes novel food as recently emerged, innovative food products produced with processes and technologies that are newly developed. To commercialize a novel food product, it must be thoroughly evaluated for safety and labeling to receive an authorization to be marketed. (European Commission n.d.)

There are many types of products that are categorized as novel food, among which cultivated meat and fermentation derived ingredients, such as from precision fermentation, are included. If the products include genetically modified ingredients, they also fall under the GMO regulation. (Executive Committee of the Codex Alimentarius Commission 2022). With technology developing and the regulations in different regions facilitating the process of going to market, the branch has grown enormously since it emerged about two decades ago. (Good Food Institute [GFI] 2026a; GFI 2026b)

4.1.1 Cultivated meat

By culturing cells isolated from an animal of agricultural importance, biomass can be produced to create meat products for human consumption. The product is highly comparable to conventional meat, allowing the consumer to choose a more sustainable alternative without altering their dietary habits. In comparison to conventional meat, cultivated meat is predicted to have a lower usage of resources, such as land, water, and feed, reduce greenhouse gas emissions by up to 92%, reduce the spread of diseases from animals to humans, and lower antibiotic resistance (Kirsch, Morales-Dalmau & Lavrentieva 2023; Good Food Institute [GFI] 2026a).

Culturing mammalian cells is not a new concept; it has long been used for tissue engineering for regenerative medicine, with a focus on biological functionality and cell viability. In cultivated meat, the aim is instead to produce desired organoleptic and nutritional qualities in a cost effective and sustainable way. The basic steps for culturing animal cells are similar for tissue engineering and for cultivated meat. First, a biopsy is taken from the animal of interest. This can be an already differentiated muscle cell or a stem cell. The cell type is isolated and developed. Further, the culture is expanded, first in laboratory scale and then in bioreactors, before being formed into an unstructured product or a structured product using a scaffold or through printing (Kirsch, Morales-Dalmau & Lavrentieva 2023).

In this production pathway, many challenges are yet to be solved, including cell selection and development, media composition, and expansion strategies. To address the engineering challenge, the ideal bioreactor design must be investigated, as factors such as culture viscosity, shear forces, and gas-liquid mass transfer rates can limit the possible cell density. Further, different process setups, such as batch and fed batch, must be investigated to increase growth rate and thereby efficiency (GFI 2026a). A representative mammalian cell line can be used for the development of the bioreactors, whose properties are more well documented, such as CHO cells. CHO cells are widely used for the production of recombinant therapeutic proteins and antibodies, and the cell medium is well developed in the pharmaceutical sector. (Zhang et al. 2024) However, the cost of cell media and bioreactors must be reduced to match the margins

in the food sector and enable economic viability for the production of cultivated meat. (GFI 2026a)

4.1.2 Precision fermentation

Traditional fermentation and biomass fermentation have existed for at least a thousand years, but recently, a more controlled and specific method using bacteria or fungi has been developed; precision fermentation. Here, an ingredient is produced rather than a whole product, as in traditional fermentation. The concentration of the product is usually lower than in traditional fermentation but often has a higher commercial value. Some emerging products are proteins, vitamins, natural pigments, fats, enzymes, and flavoring agents. (GFI 2026b) This production approach presents many environmental benefits in comparison to today's animal agriculture. The production of dairy proteins, for example, produced through precision fermentation compared to conventional products can reduce the greenhouse gas emissions by 72%, the land use by 99%, and the water consumption by 81%. (GFI Europe 2026)

Similar to cultivated meat, strain development and media formulation are important development priorities. Microbial cells are used as a production host, which usually makes cultivation easier than for cultivated meat. The strain can be genetically engineered to produce a target protein, but many naturally occurring products are also utilized. Most strains used are aerobic and have a high oxygen demand, which must be met for process efficiency. (GFI 2026b) *E. coli* is widely used in the pharmaceutical sector for the production of recombinant proteins and is well documented. Therefore, *E. coli* can serve as a representative model organism, while specifically optimized strains for production in precision fermentation are still being developed. (Glaser, Niehus, & Sha 2022)

4.1.3 Bioreactors in the field of novel food

With development in the fields of cultivated meat and precision fermentation, new developed bioreactor systems are needed. Advanced bioreactors have primarily been designed for the pharmaceutical industry, where the process control requirements are even stricter than in the food sector. The financial margins in the food sector are much smaller than in the pharmaceutical sector, meaning that higher cost efficiency in bioreactor design is of importance. Simpler fermenters from the food industry, such as for beer or dairy, do not meet the needs of aeration capacity or low shear forces for precision fermentation and cultivated meat. Therefore, bioreactors for the production of novel food products must be redesigned to meet the requirements of the industry. (GFI 2026a; GFI 2026b)

As important development is still ongoing in media and strain development in combination with bioreactor design, experimental trials across multiple scales are conducted. The seed train in bioprocesses starts at laboratory scale, to pilot scale, to ultimately reach industrial scale. At pilot scale, the process can approximate the industrial size, allowing critical parameters, such as aeration efficiency, to be optimized. Scale up parameters are often used, meaning ratios that are kept constant when going from pilot scale to industrial scale. For aeration, one common scale up parameter is the air delivery volume of liquid per minute, which is kept constant between reactor sizes. Using pilot scale reactors facilitates the evaluation of economic viability of a process and can thereby minimize the risks associated with capital investments of a full-scale industrial bioreactor. (GFI 2026b)

4.2 Aeration and sparger design

As mentioned, mammalian cells and most strains used in precision fermentation require dissolved oxygen for their growth. For bacterial cultivation, insufficient aeration is a major bottleneck in production. (GFI 2026b) Sparger designs differ widely in their oxygen transfer rates and generated shear forces. Therefore, the optimal sparger design might differ depending on the process and which strain is cultivated.

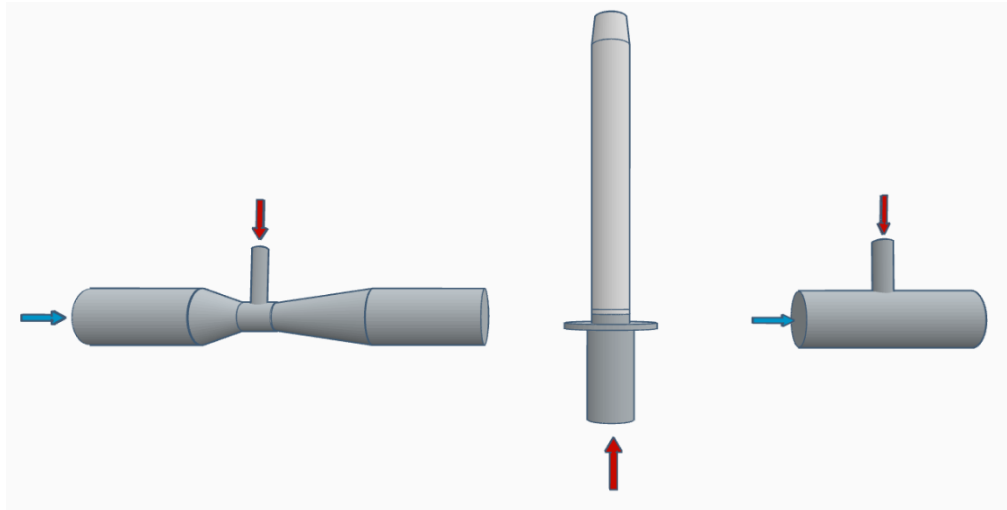


Figure 4.1. A sketch of the three aeration methods used. To the left, pipe on pipe aeration, in the middle, sintered stone, and to the right pipe on pipe. The red arrows indicate the direction of the gas flow, and the blue arrows indicate the direction of the liquid flow.

4.2.1 Venturi valve

A venturi valve has a simple design, in which a pipe converges into a throat section before diverging back to its original diameter. According to the Bernoulli principle, fluid velocity will increase as the pressure decreases in the throat section of the pipe. In the throat section, the pressure drop induces gas entrainment from a separate gas inlet pipe, usually placed vertically to the flow. This creates an intense gas-liquid interaction in the zone of low pressure after the venturi valve. It should be noted that this change in pressure can create stress on cells, which might be stronger than from the actual aeration (Huang et al. 2020). In a venturi aeration system, the most important parameters include the cone angle, the gas-liquid ratio, and the throat dimensions (Puri, Sihag & Thakur 2023).

Recently, applications of venturi valves gained increased interest, and their performance needs evaluation. Some of the properties that make venturi valves an interesting option when choosing aeration method in the biotech and chemical industry, as well as for water treatments and fish farming, are that venturi valves have been shown to have low power consumption compared to other sparging methods. It has a simple design with no moving parts, facilitating installation and maintenance. By adjusting the liquid and gas flow rates and the design of the valve, the bubble size can be controlled (Huang et al. 2020).

4.2.2 Sintered stone

Porous spargers are commonly produced from metal, ceramics, or glass. When made of metal, they are often referred to as sintered stones or sintered spargers. Depending on the surface area and pore structure, small bubbles are created, but also a large pressure drop in the flow which

might limit the gas throughput. Therefore, porous spargers are mostly used in small scale set-ups, usually without agitation. Another concern with porous spargers is that cells can get trapped in the pores, causing blockage and making them hard to clean. (Stanbury, Whitaker & Hall 2017a).

4.2.3 Pipe on pipe

For aeration in reactors with agitation, gas can be introduced into the liquid phase through a perforated pipe, as an orifice sparger. The perforated pipe is typically arranged in a cross or circular configuration underneath the impeller. Orifice spargers can also be used in unagitated environments, for example in yeast manufacturing or for single cell proteins in airlift reactors. The hole diameter is often recommended to be at least 6 mm to avoid blockage and a limiting pressure drop (Stanbury, Whitaker & Hall 2017a).

4.2.4 Determination of the oxygen transfer rate using the dynamic method

To be able to optimize and compare the efficiency of aeration in a bioreactor, the oxygen transfer must be quantified. There are many methods to investigate the oxygen transfer rate, but one common comparable value used to compare aeration capacity is the volumetric mass transfer coefficient (k_La). The value depends on reactor design and operating conditions, and although usually combined, k_La consists of two parts. k_L is the gas-liquid oxygen transfer resistance, and a is the area of the bubble surface, depending on the number of bubbles, their size, and residence time (Stanbury, Whitaker & Hall 2017b).

There are multiple methods to determine the k_La value for a certain process, with one of them being the dynamic method. By monitoring changes in dissolved oxygen (DO) concentration using a DO sensor, the dynamic method allows estimation of the k_La value. Since the oxygen transfer rate is specific to the conditions at which the process is running, they must be kept constant throughout the measurements. Equation 1 shows that by creating an unsteady state, the oxygen transfer rate can be quantified using mass balance equations for the dissolved oxygen concentration (Liu 2020).

$$\frac{dC_{O_2}}{dt} = OTR - OUR \quad (1)$$

The unsteady state can be created by degassing the liquid in the reactor by either stopping aeration, allowing the cells to consume the dissolved oxygen, or by sparging nitrogen gas into the liquid. The k_La value is then determined by sparging air into the liquid at a constant flow rate, which increases the oxygen concentration in the liquid. If the degassing did not reach a critical level for the cells, it is assumed that the cell growth is not significantly affected. In a steady state, the sparging is balanced with oxygen consumption by the cells. In a biological system, Equation 1 can be rewritten into Equation 2 (Liu 2020).

$$\frac{dC_{O_2}}{dt} = k_La * (C_{O_2}^* - C_{O_2}) - q_{O_2} * x \quad (2)$$

Equation 2 can be arranged as presented in Equation 3 when the system is in a steady state, meaning that the oxygen transfer (left-hand side) and oxygen consumption (right-hand side) are equal.

$$k_La * (C_{O_2}^* - C_{O_2}) = q_{O_2} * x \quad (3)$$

k_La is of high importance, as it represents the reactor's ability to supply oxygen to meet the demand of the cells. To determine the value of k_La more accurately, aeration can be done in a

non-biological system, meaning that there is no oxygen consumption, creating an unsteady state. In this case, Equation 2 can be linearized into Equation 4, allowing the left-hand side of the equation to be plotted as a function of time, where k_{La} is the slope of the graph.

$$\ln(C_{O_2}^* - C_{O_2}) = -k_{La} * t \quad (4)$$

4.2.5 Oxygen requirement of cell cultures

The cell oxygen demand can differ in magnitude and depends on the type of cells, their growth phase, and the cell density. (Doran, Morrissey & Carlson 2025a) To make a process profitable, the highest possible cell concentration should be supported by the aeration. In Table 4.1 typical oxygen uptake, high cell concentrations, and oxygen demand are shown for *E. coli* and CHO cells. CHO cells have a significantly lower oxygen demand than *E. coli*. In Table 4.1, oxygen concentration at saturation is also shown, which is dependent on the media composition. The oxygen concentration at saturation is based on processes run at 30°C for *E. coli* and at 37°C for CHO cells. It is estimated that oxygen solubility is 10% lower than in pure water due to solute effects. Since media and processes differ, these numbers are approximate. (Doran, Morrissey & Carlson 2025a). Note that the *E. coli* is measured in g dry cell weight (dcw)/l and CHO cells in cells/l.

Table 4.1. Oxygen requirement and cell densities for different cell cultures. Dissolved oxygen concentration characteristics in the medium for these cell cultures are also shown.

	<i>E. coli</i>	CHO cells
Oxygen requirement	8.5 mmol/g dcw*h (Doran, Morrissey & Carlson 2025a)	2.9 *10 ⁻¹⁰ mmol/cell*h (Doran, Morrissey & Carlson 2025a)
Cell density	25 g dcw /l (Doran, Morrissey & Carlson 2025a)	10 ⁹ cells/l (Doran, Morrissey & Carlson 2025a)
Dissolved oxygen concentration at saturation in cell media	7.2 mg/l	6.6 mg/l
Typical operating of dissolved oxygen concentration	30 % (Glaser, Niehus, & Sha 2022)	40 % (Mirro 2011)

4.3 Shear stress

When designing a bioreactor for sensitive cultures, the shear forces generated in the reactor are a key factor to consider. In reactors with agitation, like in a stirred tank reactor, agitation is the dominant source of shear forces. Shear forces by agitation can be managed by varying the agitation speed and the impeller design and size. In reactors without agitation, as in an airlift reactor, the highest shear forces come from aeration. Shear forces from aeration can be regulated by adjusting the airflow and changing the sparger design. Since shear forces and aeration are highly interdependent, both must therefore be considered when deciding on operating conditions. (Stanbury, Whitaker, & Hall 2017a; Esperança et al. 2020)

There is no single established model that can quantify all shear forces in a bioreactor, but as mentioned, the main sources of shear are agitation and aeration, in that order. Therefore, the two factors can be investigated to define the operation conditions to avoid lethal conditions for the cell culture. Ideally, shear forces from both agitation and aeration should be investigated

using a CFD model, but such models must be highly complex and detailed to make accurate estimations. Instead, calculations on shear forces from different sources can create limiting operating conditions.

4.3.1 Quantification of shear forces from agitation

When scaling agitation in bioreactors, two commonly used scale-up parameters used are power density and impeller tip speed. These two factors can be used to estimate the created shear forces by agitation. Power density is expressed as the power input from agitation divided by the fluid volume over which the energy is assumed to be distributed, as shown in Equation 5. (Muralidharan, Bolduc & Davis 2024)

$$\frac{P}{V} = \frac{\rho N_p N_i^3 D_i^5 n_i}{V} \quad (5)$$

The impeller power number, N_p , in Equation 5 is specific to a certain impeller design. The shear from the agitation is greatly impacted by the design of the impeller, where a marine impeller creates considerably lower shear force than a Rushton impeller. For multiple impellers, the total power input can be approximated as the sum of each impeller. (Doran, Morrissey & Carlson 2025b)

A limitation of this approach for estimating shear is that the power is assumed to be distributed uniformly throughout all the fluid in the reactor but is in reality concentrated near the impeller. Therefore, the impeller tip speed is used to represent the local maximum shear, as seen in Equation 6.

$$v_i = \pi D_i N_i \quad (6)$$

A disadvantage of this model is that the equation for impeller tip speed is independent of the impeller design, which does not consider that different impeller designs create different amounts of cavitation.

4.3.2 Quantification of shear forces from aeration

Shear forces generated by sparging can be divided into three main phenomena, and depending on the operating conditions and the sparger used, the dominant mechanism can vary. The three phenomena are bubble entrance, bubble ascending, and bubble rupture. The shear forces from these three phenomena can be calculated separately to know how they compare in magnitude to each other. Out of the three phenomena, bubble ascending is the one creating the least shear forces, and it is therefore of higher relevance to study gas entrance and bubble rupture. (Neunstoecklin et al. 2015)

For bubble entrance, the shear can be estimated to be proportional to the gas entrance velocity (GEV) at which the gas passes through the hole of the sparger and becomes a bubble in the liquid. As Equation 7 shows, GEV is a function of the hole size, number of holes, and air flow.

$$GEV = \frac{Q_g}{n_h * \frac{\pi d_h^2}{4}} \quad (7)$$

Further, shear forces are generated when the bubbles from aeration rupture at the liquid surface. A model based on empirical trials estimates the relation in Equation 8 between bubble diameter

and energy dissipation rate (EDR) from the rupture of bubbles. EDR can be used as an indicator of local shear forces. (Muralidharan, Bolduc & Davis 2024)

$$EDR = e^{-(194.34*(\log(d_b*10^2))^3 + 217.02*(\log(d_b*10^2))^2 + 129.07*(\log(d_b*10^2)) - 19.599)} \quad (8)$$

A disadvantage of this model is that it does not take into account the total amount of bubbles rupturing at the surface, meaning that the forces created by the bubbles rupturing are assumed not to be accumulative. Models including the cumulative factor of the air bubbles rupturing are very complex and would need a precise CFD model. Another disadvantage of this model is that if the bubble diameter is not known, it must be estimated, introducing additional uncertainty.

4.3.3 Shear stress effect on cells

Bacterial cells are much more resistant to shear forces than mammalian cells. One of the reasons for this is the size of the cell, but mammalian cells are also inherently more sensitive. (Stanbury, Whitaker & Hall 2017b) For all mammalian cell lines, shear forces in the upscaling of bioreactors are among the most critical factors. Although there are differences between mammalian cell lines, even relatively small shear forces can cause metabolic changes or cell death. Investigating the maximum amount of shear forces becomes more complex since the sensitivity also depends on the growth phase of the cell and the media composition. (Stanbury, Whitaker, & Hall 2017a)

Shear forces high enough to affect unicellular yeast and bacteria are unlikely to be created in bioreactors run under typical operating conditions. Although for filamentous fungi, high shear forces from agitation can affect morphology and disrupt cell aggregates (Doran, Morrissey & Carlson 2025b). Bacterial cultures often have a high oxygen demand to reach high productivity. As aeration and shear forces are closely related and bacterial cultures are less sensitive to shear stress, bacterial processes are often operated under high shear conditions. (Stanbury, Whitaker & Hall 2017b) It is important to note that shear forces can affect cell culture in more subtle ways than just being lethal to the cells. Cell growth and product synthesis can be inhibited, and extracellular compounds can be affected by harsh growth conditions. (Doran, Morrissey & Carlson 2025b)

To determine the detrimental level of shear forces for different cell cultures used in cultivated meat and precision fermentation under normal operation conditions in a bioreactor, data has been compiled and is shown in Table 4.2. The absence of defined shear limits for *E. coli* is based on the fact that typical operating conditions do not generate shear forces large enough to substantially affect their growth. (Stanbury, Whitaker & Hall 2017b) These thresholds define the upper limits on agitation and aeration as operating conditions for these cell cultures.

Table 4.2. Detrimental amount of shear forces for different quantities.

	<i>E. coli</i>	CHO cells
General shear sensitivity	Low (Stanbury, Whitaker & Hall 2017b)	High (Stanbury, Whitaker & Hall 2017b)
<i>P/V</i>	- (Stanbury, Whitaker & Hall 2017b)	35 W/m ³ (Muralidharan, Bolduc & Davis 2024)
<i>v_i</i>	- (Stanbury, Whitaker & Hall 2017b)	0.6 m/s (Muralidharan, Bolduc & Davis 2024)
<i>GEV</i>	- (Stanbury, Whitaker & Hall 2017b)	60 m/s (Muralidharan, Bolduc & Davis 2024)
<i>EDR</i>	- (Stanbury, Whitaker & Hall 2017b)	10 ⁶ -10 ⁸ W/m ³ (Muralidharan, Bolduc & Davis 2024)

4.4 The bioreactors used in this report

The pilot bioreactor setup used in this report was designed by Curve Biotech to bridge the gap between laboratory scale and industrial scale for the novel food industry. By taking inspiration from both bioreactors in the pharmaceutical sector and from yeast propagators in the brewing industry, the reactors are cost-efficient yet modular. They are designed to generate low shear stress suitable for the cultivation of mammalian cells, while having multiple aeration systems to meet the oxygen requirements for a demanding microbiological process. Since processes in these reactors are made to be developed to reach industrial size, they have to be efficient to be economically feasible, including the aeration setup, energy efficiency, and operational labor. The setup consists of two bioreactors with operational volumes up to 310 liters, including sensors for pH, dissolved oxygen, pressure and more. In this report, only one of the reactors will be used, which has an agitator with two marine impellers installed with a maximum agitation rate of 17 700/s. Percentages of agitation are based on the maximum value.

The bioreactor used can be aerated through sintered stone, pipe on pipe aeration, and through a venturi valve. Both the sintered stone and the pipe on pipe design are placed in the bottom of the bioreactor. The venturi valve is placed in an external loop in the bioreactor, through which liquid is circulated. Since the liquid is pumped through the separate loop, the liquid flow can be varied by regulating the pump's settings.

As described in Section 4.2.1 the throat section creates a local pressure drop after the venturi valve. To regulate this, a regulation valve is placed further down in the flow, to increase the pressure and intensity of the gas-liquid interactions. The already found optimal pump setting and flow regulation are used in the experimental trials of this thesis.

5 Material and Methods

5.1 k_{La} determination using the dynamic method

As described in Section 4.2.4, the dynamic method to determine k_{La} is based on the mass balance of dissolved oxygen in the reactor by reaerating a degassing liquid. k_{La} was determined for different sparger designs, flows of air and different agitation speeds in a non-biological system in one of Curve Biotech's bioreactors. For all trials, the same reactor was used with 310 l water at room temperature ($20^{\circ}\text{C} \pm 2^{\circ}\text{C}$) and at atmospheric pressure. The DO sensor (Mettler Toledo, InPro 6850i) was positioned at mid-height along the reactor wall. The DO sensor was calibrated in water prior to the experiments. All trials were made in duplicates, apart from trials with pipe on pipe aeration at 50% agitation.

For degassing, the dissolved oxygen was decreased using nitrogen purging. In all trials, nitrogen gas at a pressure of 1 bar was sparged into the reactor using a sintered stone until the DO reached 4 mg/l. The nitrogen purging was then turned off for the system to stabilize. A visual inspection was performed to confirm that most of the nitrogen bubbles in the reactor had vanished. For trials with agitation, the agitation was turned on 10 minutes prior to the aeration.

k_{La} at different air flows was determined for three different spargers, sintered stone, pipe on pipe design, and venturi valve. The experimental conditions at which trials were conducted are shown Table 5.1. The design of the spargers is described further in Section 4.2. When the DO had stabilized after the deaeration, aeration at a certain air flow was started using one of the mentioned spargers. The sintered stone is placed directly into the bottom of the reactor, and the pipe on pipe aeration is also in direct contact with the reactor volume. For trials using the pipe on pipe system, agitation at different speeds was tried. The agitation was kept at the same determined value throughout the whole trial. For trials with the venturi valve, the water was circulated through an external loop containing the venturi valve, with a low shear force pump flow at 3.5 m³/h. The DO was recorded with one minute intervals until oxygen saturation was reached. A display of the operating conditions at which trials were conducted is shown in Table 5.1. The percentage of agitation is based on the maximum value of 4.9/s, meaning that 30 % agitation is 1.5 /s and 50 % agitation is 2.5 /s.

Table 5.1. Operating conditions under which experimental trials were conducted. All trials were made in duplicate, apart from pipe on pipe 50% agitation.

Air flow	Sintered stone No agitation	Pipe on pipe No agitation	Pipe on pipe 30 % agitation	Pipe on pipe 50 % agitation	Venturi valve No agitation
0.5	X	X	X		X
1	X	X	X	X	X
3	X	X	X		
5	X	X	X		

Further, control trials measuring DO were made where water was left undisturbed for 2 hours. Control trials of agitation and circulation in the external loop, respectively, were made for 2 hours without aeration.

To calculate the k_{La} based on the results, Equation 4 was used, where 9.1 mg/l was used as the maximum saturation. (Doran, Morrissey & Carlson 2025a) The natural logarithm of the oxygen

concentration subtracted from the maximum oxygen concentration was plotted against time, and a linear regression was applied. The absolute value of the slope is the k_{LA} value, as is shown in Equation 4. k_{LA} was then normalized by dividing it by the gas flow rate to evaluate oxygen transfer efficiency.

5.2 Shear forces from agitation

Shear forces from agitation were determined using two different methods, where one measures the average shear and the other the local maximum shear. The values used are the ones defined by the bioreactors used. The following calculations are used to estimate the shear forces generated by agitation while aerating, corresponding to the experimental operating conditions in the previous section.

To determine the average shear forces in the reactor, Equation 5 was used.

$$\frac{P}{V} = \frac{\rho N_p N_i^3 D_i^5 n_i}{V} \quad (5)$$

In the bioreactor setup, two marine impellers are installed. The power number, N_p , used is therefore 0.35. (Doran, Morrissey & Carlson 2025b). Each impeller has a diameter of 0.16 m. The density is the one for water (1000 kg/m³). The reactor volume is 310 l.

For estimations of the local maximum shear force by impeller tip speed, Equation 6 is used.

$$v_i = \pi D_i N_i \quad (6)$$

For both Equation 5 and Equation 6, the agitation speed, N_i is varied, and the power density and impeller tip speed are calculated. The maximum agitation speed to avoid a detrimental effect on the cell culture can be determined using the data in Table 4.2.

5.3 Shear forces from aeration

To compare shear forces from aeration, two representative phenomena will be compared, the gas entrance, GEV , and the bubble rupture measured by EDR . These two will be assessed based on varying operational conditions and the technical design of the spargers. The following calculations are used to estimate the shear forces generated by aerating, corresponding to the experimental operating conditions in the earlier section.

GEV is calculated using Equation 7 as previously shown.

$$GEV = \frac{Q_g}{n_h * \frac{\pi d_h^2}{4}} \quad (7)$$

As described in Section 5.1, experimental trials are performed using the three aeration designs, pipe on pipe, sintered stone, and venturi valve. Therefore, the variables n_h (number of holes) and d_h (hole diameter) will be defined by the design of these three aeration methods. The values used for the calculations are shown in Table 5.2. During operation, the air flow can be varied, and the calculation will therefore be based on varying air flow for the three aeration methods.

Table 5.2. Technical information on the number of holes and hole diameter in the sparger, which are used in the equations on shear forces from aeration.

Aeration method	Number of holes	Hole diameter
Sintered stone	28.5*10 ⁶	20 µm
Pipe on pipe	1	2.5 cm
Venturi valve	1	1.5 cm

For the shear forces created by air bubbles rupturing at the surface, Equation 8 will be used.

$$EDR = e^{-(194.34*(\log(d_b))^3+217.02*(\log(d_b))^2+129.07*(\log(d_b))-19.599)} \quad (8)$$

The equation uses bubble diameter and calculates energy dissipation. Air bubbles are estimated to be 10 times larger than the aeration hole. This is an assumption that gives an estimation of magnitude based on the fact that the bubble from a sparger can be estimated to be 10-100 times larger than the sparger hole, and since smaller bubbles create larger energy dissipation, using the lowest value in the range gives a more conservative estimate. (Stanbury, Whitaker & Hall 2017a).

5.4 Operational window

Operational windows are constructed for two representative cultures; CHO cells and *E. coli*, and four aeration methods; venturi valve, pipe on pipe aeration with and without agitation, and sintered stone. The lower limit will be set by the oxygen requirement, and the upper limit by the maximum shear forces. This allows the identification of a framework for operating conditions, where the oxygen demand is met, while remaining below detrimental shear conditions.

6 Results

6.1 k_{La} determination using the dynamic method

A total of 29 trials were made, duplicates of the conditions shown in Table 5.1, except for pipe on pipe aeration with 50% agitation, which was only performed once. Controls of when water was left undisturbed, agitated, and circulated, respectively, for 2 hours without aeration all show that there was no increase in DO that could influence the results of the aerated trials.

As all datasets were processed in the same way, one representative example of data handling is shown, and only the final results from the other trials. The following example is a trial using the pipe on pipe aeration method with 30% agitation. In Table 6.1, the measured DO values are shown, with their linearized form.

Table 6.1. An example of DO results from k_{La} trials according to the dynamic method using the pipe on pipe aeration method with 30% agitation. At minute 1 aeration was started and DO was tracked.

Time (min)	DO (mg/l)	$\ln(9.1-DO)$
0	3.58	1.708378
1	3.63	1.699279
2	4.12	1.60543
3	5.12	1.381282
4	6.00	1.131402
5	6.7	0.875469
6	7.15	0.667829
7	7.59	0.41211
8	7.93	0.157004
9	8.17	-0.07257
10	8.34	-0.27444
11	8.47	-0.46204
12	8.58	-0.65393
13	8.73	-0.99425
14	8.80	-1.20397
15	8.87	-1.46968
16	8.91	-1.66073
17	8.94	-1.83258
18	8.97	-2.04022
19	8.99	-2.20727
20	9.01	-2.40795
21	9.04	-2.81341
22	9.05	-2.99573
23	9.06	-3.21888
24	9.06	-3.21888

The results in Table 6.1 are visualized in Figure 6.1 and Figure 6.1. Figure 6.1 show the increase in DO after aeration was started and approached saturation after 25 minutes.

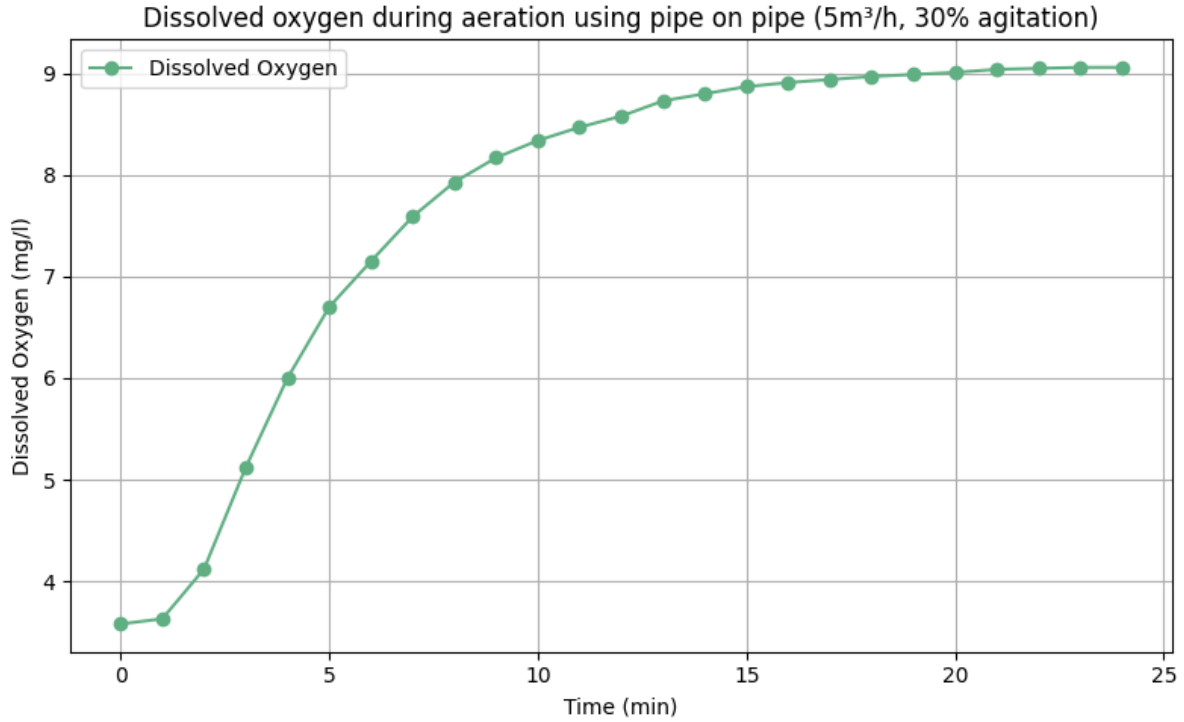


Figure 6.1. Dissolved oxygen over time after aeration was started at minute 1 using the pipe on pipe method with 30% agitation.

In Figure 6.2 the linearized results in Table 6.1 are plotted against time. The first and last data points are removed from the regression since it was not part of the aeration process. According to Equation 4, the absolute value of the slope of the linear regression corresponds to $k_L a$.

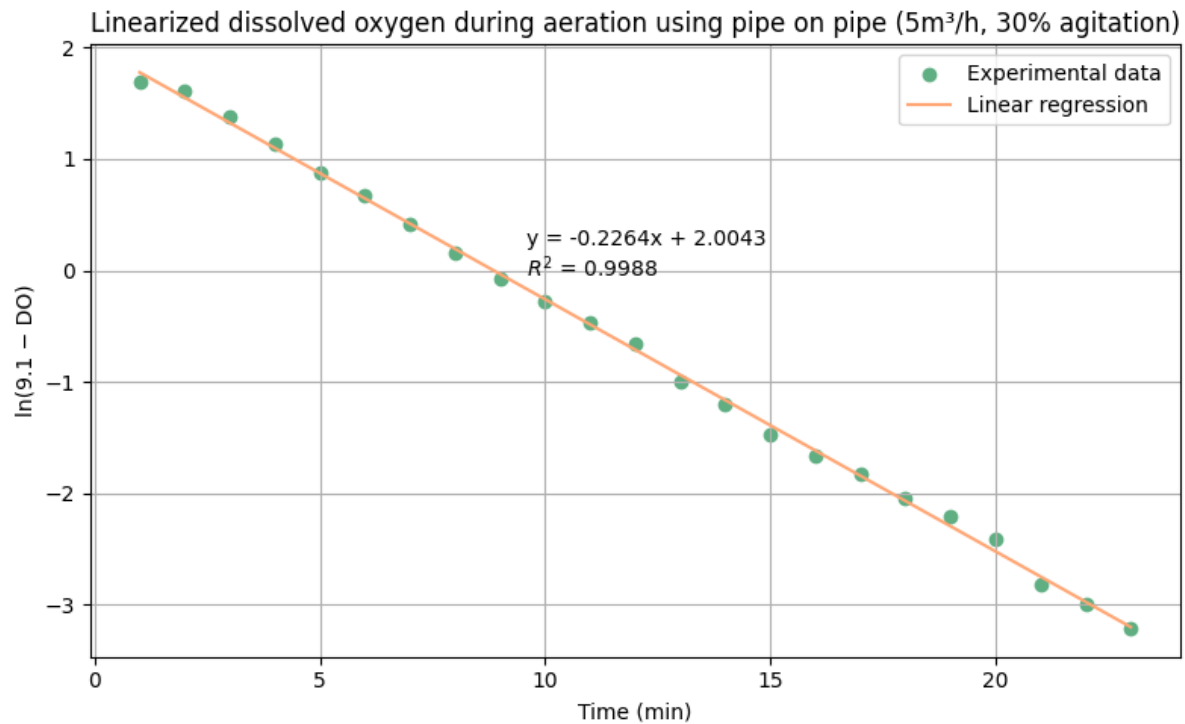


Figure 6.2. Linearized DO over time after aeration was started at minute 1 using the pipe on pipe method with 30% agitation. A linear regression and its equation are also shown in the graph.

Figures 6.3-6.5 show the raw data results from aeration with sintered stone, pipe on pipe aeration, and venturi valve.

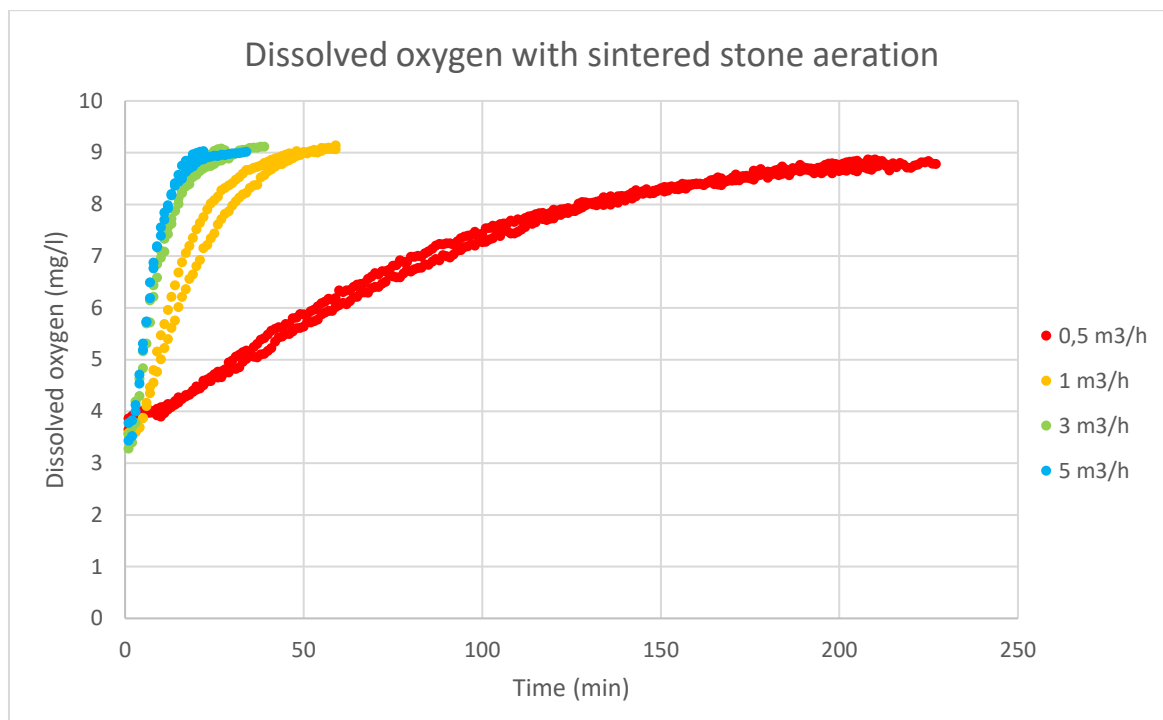


Figure 6.3. Dissolved oxygen over time when sparging with a sintered stone with different air flows.

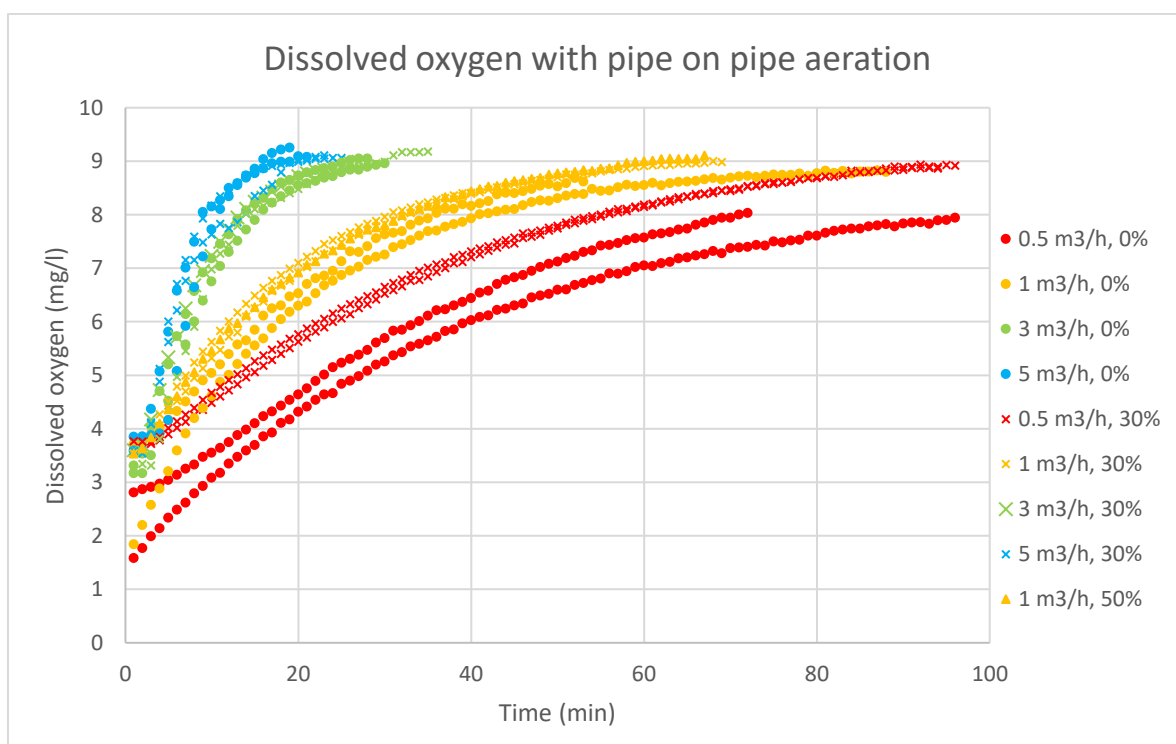


Figure 6.4. Dissolved oxygen over time when sparging with a pipe on pipe aeration with different air flows and different agitation rates (indicated by percentage).

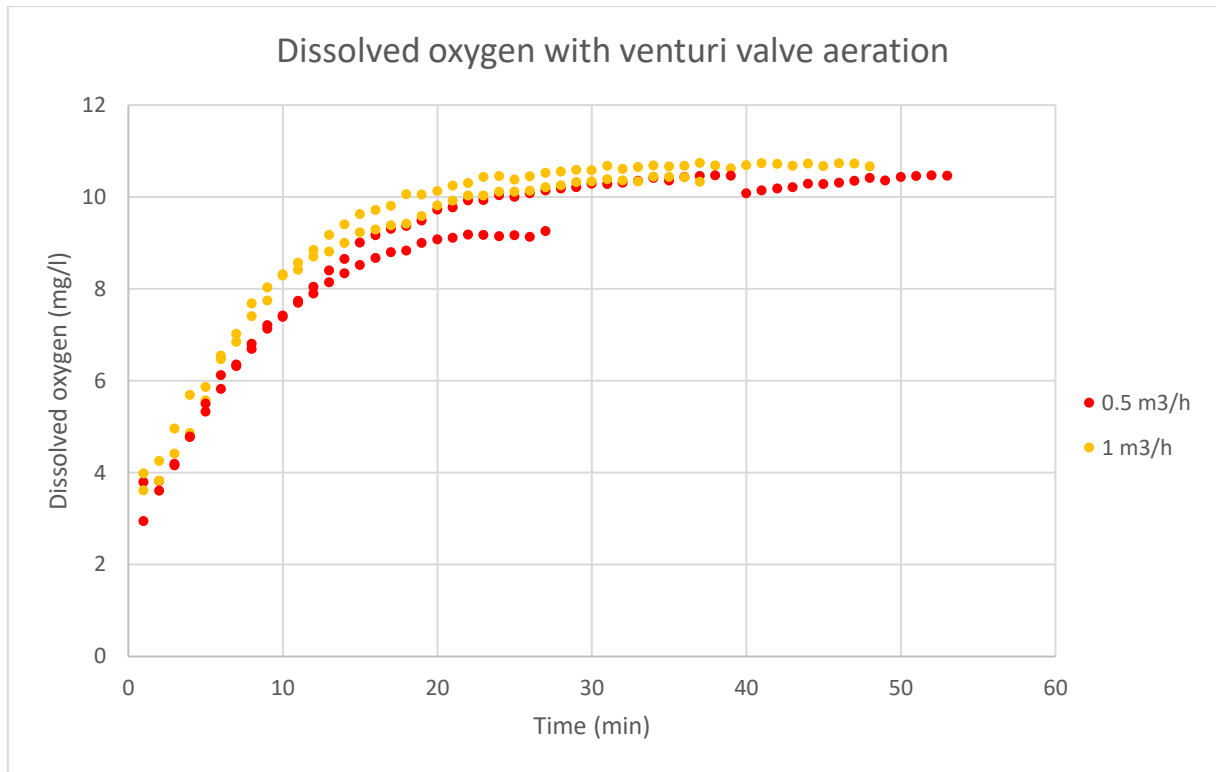


Figure 6.5. Dissolved oxygen over time when sparging with venturi valve with different air flows.

Using the same procedure as explained previously, all 29 data sets were processed. The resulting k_{La} values calculated from the data in Figure 6.3-6.5, are shown in Table 6.2-6.6, and summarized in Figure 6.6.

Table 6.2. k_{La} values (/min) for all trials using the sintered stone for aeration without agitation based on the dynamic method. Different air flows were investigated, which are shown in the table.

Air flow	Sintered stone no agitation			
	Trial 1	Trial 2	Average	Standard deviation
0.5	0.013	0.014	0.014	0.000707
1	0.075	0.088	0.082	0.009334
3	0.159	0.127	0.143	0.022981
5	0.232	0.166	0.199	0.046669

Table 6.3. k_{La} values (/min) for all trials using the pipe on pipe method for aeration without agitation based on the dynamic method. Different air flows were investigated, which are shown in the table.

Air flow	Pipe on pipe no agitation			
	Trial 1	Trial 2	Average	Standard deviation
0.5	0.026	0.017	0.022	0.006505
1	0.04	0.050	0.045	0.007071
3	0.135	0.168	0.151	0.023122
5	0.299	0.235	0.267	0.045467

Table 6.4. k_{La} values (/min) for all trials using the pipe on pipe method for aeration with 30% agitation based on the dynamic method. Different air flows were investigated, which are shown in the table.

Air flow	Pipe on pipe 30% agitation			
	Trial 1	Trial 2	Average	Standard deviation
0.5	0.036	0.037	0.037	0.000707
1	0.055	0.058	0.056	0.001697
3	0.145	0.154	0.149	0.006293
5	0.227	0.202	0.214	0.017961

During trials with 50% agitation, significant gas holdup around the shaft was observed, and no further trials under these conditions were conducted.

Table 6.5. k_{La} values (/min) for all trials using the pipe on pipe method with 50% agitation based on the dynamic method. Different air flows were investigated, which are shown in the table.

Air flow	Pipe on pipe 50% agitation
	Trial 1
1	0.065

Table 6.6. k_{La} values (/min) for all trials using the venturi valve for aeration without agitation based on the dynamic method. Different air flows were investigated, which are shown in the table.

Air flow	Venturi valve no agitation			
	Trial 1	Trial 2	Average	Standard deviation
0.5	0.189	0.184	0.187	0.003535
1	0.290	0.238	0.264	0.036769

Figure 6.6 shows the results of k_{La} values for different aeration methods, and air flows from Table 6.2-6.6. Linear regressions are applied for all operating conditions except for the trial with 50% agitation, which are shown with their equations. Note that the relationship is assumed

to be a regression through origin, meaning that the k_{La} at no gas flow is assumed to be zero, confirmed by the control trials showing no increase in DO.

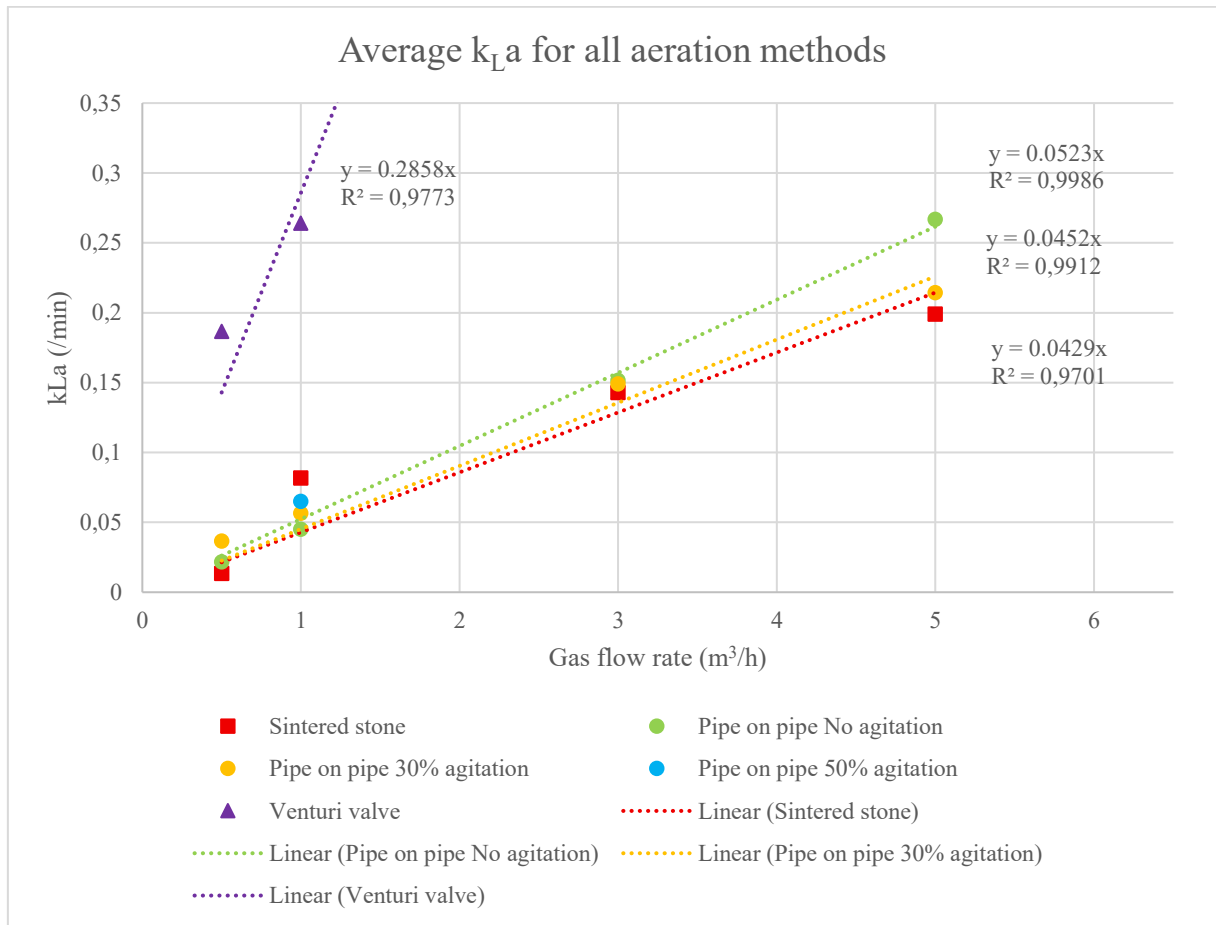


Figure 6.6. k_{La} value of all trials using different aeration methods and different airflow. For aeration methods where different air flows are used, a regression is inserted with its equation.

To further investigate the oxygen transfer efficiency, k_{La} was divided by the air flow, as is shown in Figure 6.7.

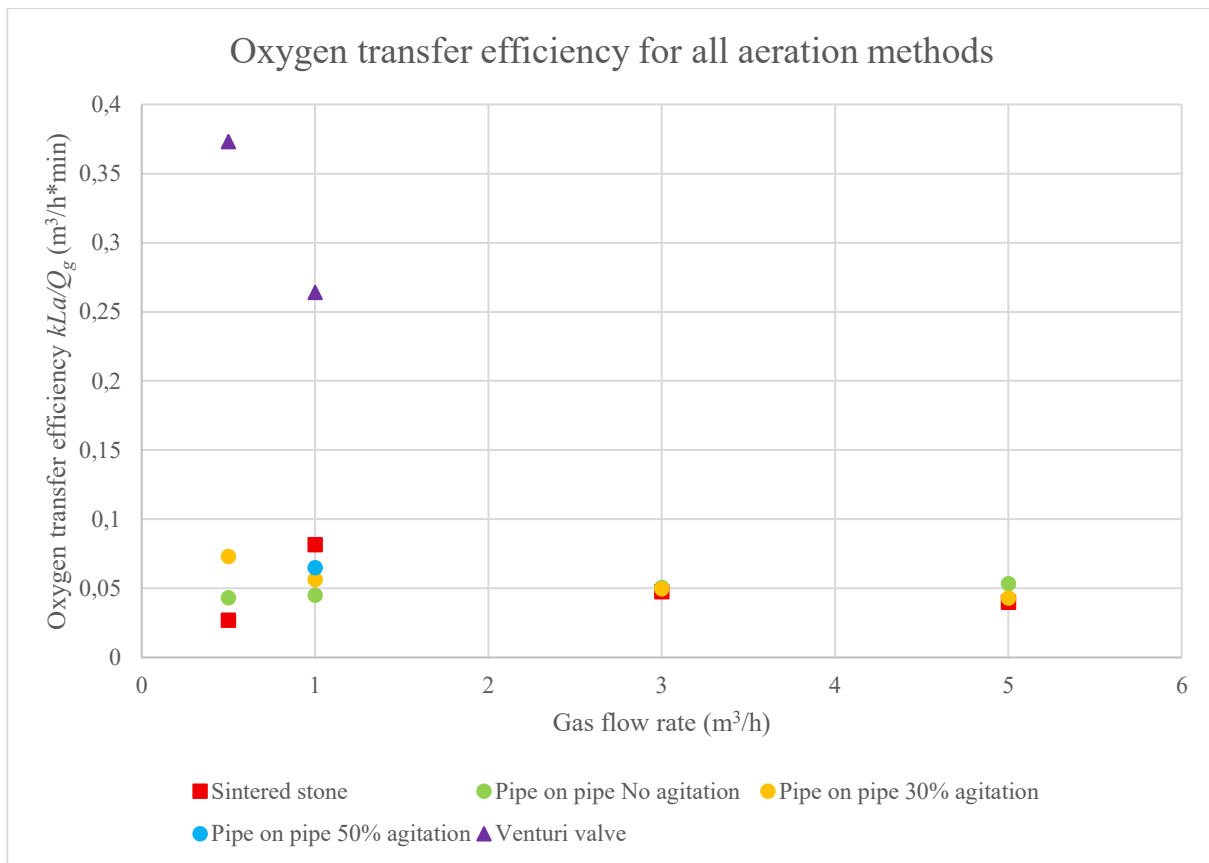


Figure 6.7. k_{La} is divided by gas flow to show oxygen transfer efficiency.

6.2 Shear forces from agitation

The results based on Equation 5 are shown in Figure 6.8, where the agitation rate is varied, resulting in different power density values for the 310 l bioreactor. The horizontal line shows the lethal level of power density for CHO as described in Table 4.2, which is calculated to be reached at an agitation of 5.29 /s.

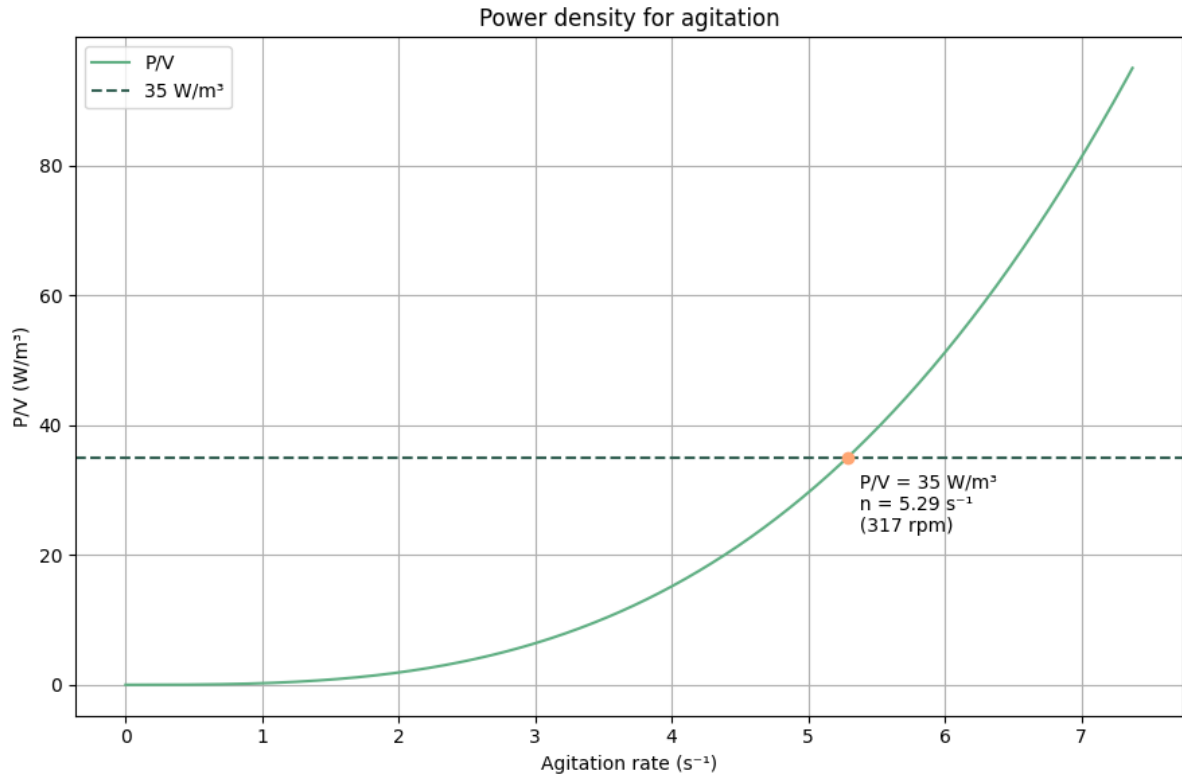


Figure 6.8. The power density when agitating a 310 l bioreactor is shown. The dashed line shows the lethal level of power density for CHO cells.

The results from Equation 6 are shown in Figure 6.9, where it is shown how agitation speed relates to impeller tip speed. The dashed line shows the lethal tip speed for CHO cells, which is reached at an agitation of 1.19/s.

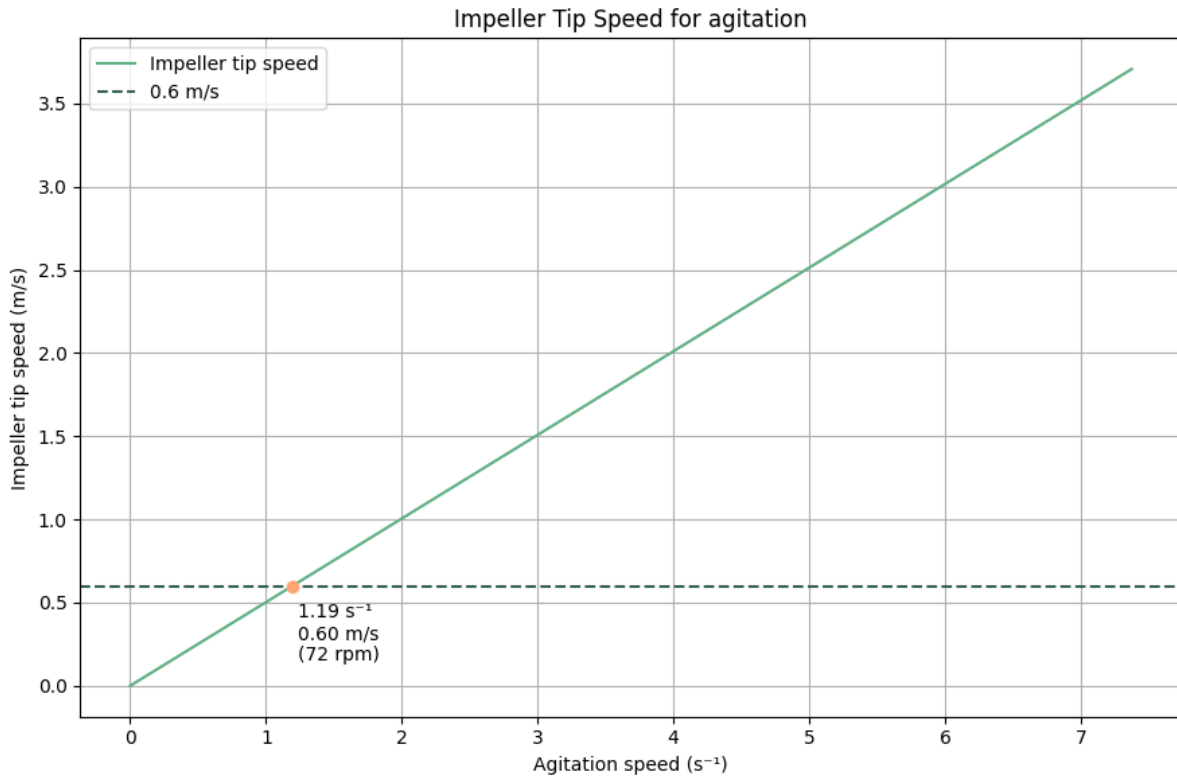


Figure 6.9. The impeller tip speed when agitating a 310 l bioreactor is shown. The dashed line shows the lethal tip speed for CHO cells.

6.3 Shear forces from aeration

Figure 6.10 shows GEV relating to gas flow for the three sparger methods pipe on pipe, sintered stone, and venturi valve based on Equation 7, and the technical data in Table 4.2 is shown. A dashed line shows the lethal GEV for CHO cells at 60 m/s, intersecting with the curves. The venturi valve reaches critical GEV of 60 m/s at the lowest gas flow, at 38.24 m^3/h , whereas the valve for pipe on pipe reaches this value at 106.11 m^3/h . For the sintered stone, critical GEV is reached outside of typical operating conditions.

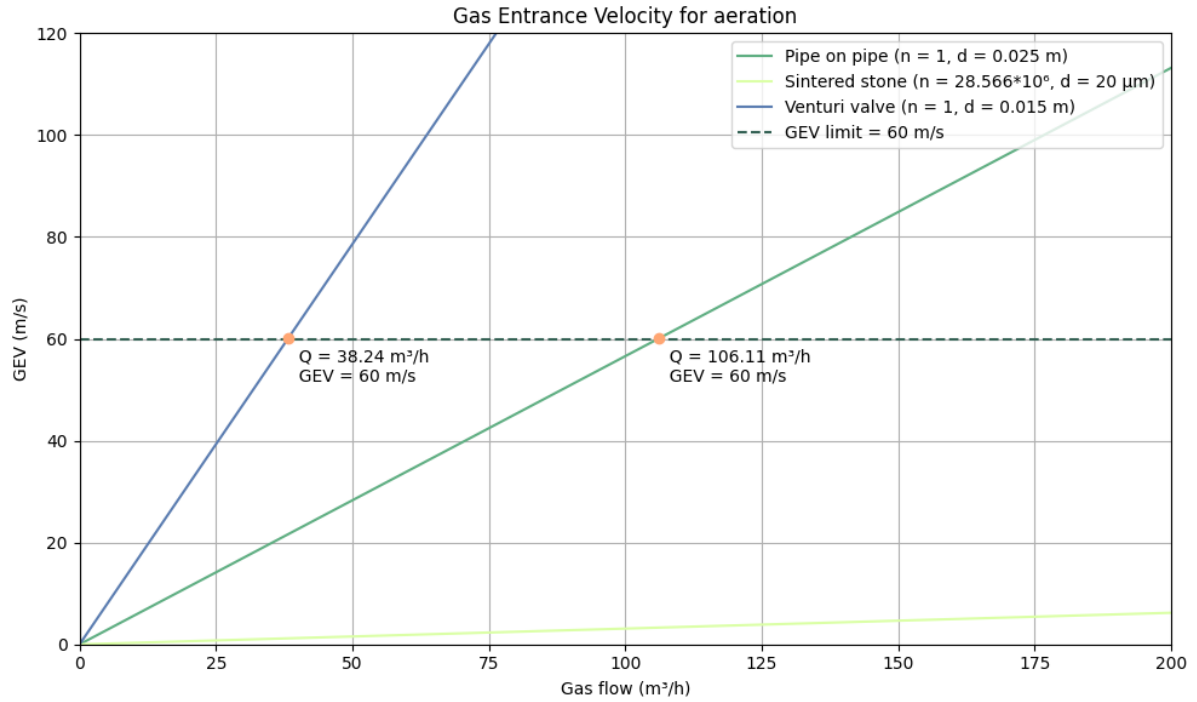


Figure 6.10. Gas entrance velocity as a function of gas flow for three different sparger designs is shown. A horizontal dashed line shows the lethal gas entrance velocity limit for CHO cells.

Figure 6.11 shows *EDR* associated with bubbles rupturing at the liquid surface as a function of bubble size, which shows that smaller bubbles create larger energy dissipation. A dashed line at 10^6 W/m^3 shows the lethal *EDR* for CHO cells, which is reached at a bubble size of approximately 0.331 cm.

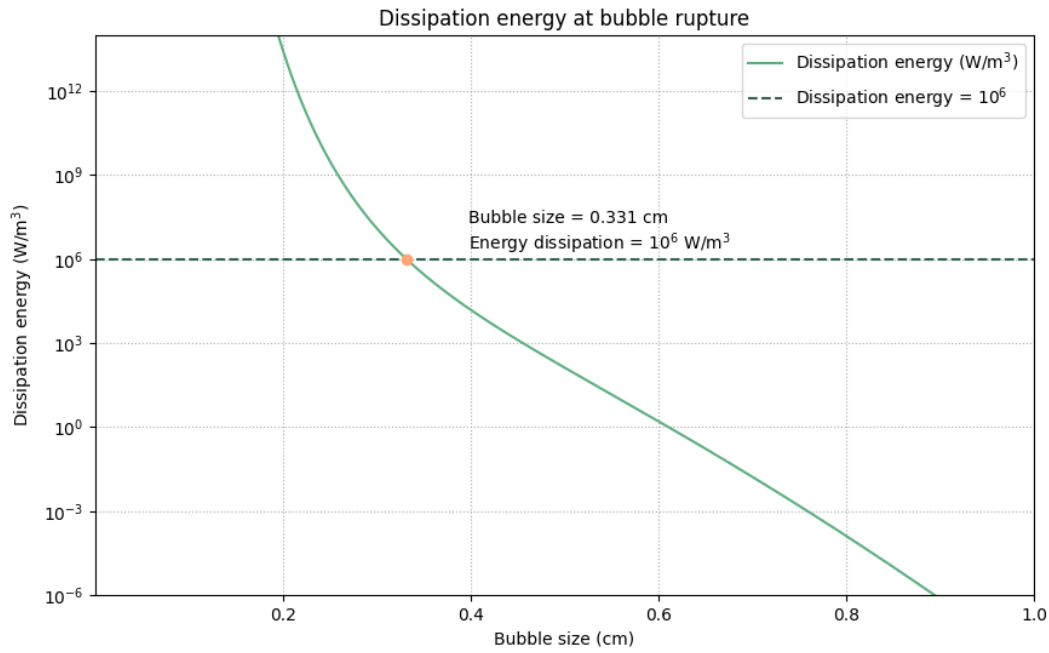


Figure 6.11. Dissipation energy as a function of bubble size. A horizontal dashed line shows the dissipation energy limit for CHO cells.

6.4 Operational window

To create an operational window, the aeration must meet the oxygen requirements of the cell culture, while not exceeding critical shear stress levels.

6.4.1 Gas flow needed based on the oxygen requirement

Using the data in Table 4.1, the k_La needed at a certain cell density, oxygen consumption, and oxygen concentration can be calculated in a steady state. First, values from Table 4.1 are inserted in Equation 3 for CHO cell culture. The molar mass of oxygen is used as 31.998 mg/mmol.

$$k_La = \frac{2.9 * 10^{-10} \frac{\text{mmol}}{\text{cell} * \text{h}} * 31.998 \frac{\text{mg}}{\text{mmol}} * 10^9 \text{cell/l}}{\left(6.6 \frac{\text{mg}}{\text{l}} - 6.6 \frac{\text{mg}}{\text{l}} * 0.4\right)} = 2.34/\text{h} = 0.039/\text{min} \quad (9)$$

The resulting k_La value is 2.34 /h (0.039 /min). Using this needed k_La value, the gas flow needed for different sparging methods can be found using the regression in Figure 6.6. These values are shown in Table 6.7, together with the linear regressions.

For *E. coli*, the values from Table 4.1 are inserted similarly as follows.

$$k_La = \frac{8.5 \frac{\text{mmol}}{\text{g dcw} * \text{h}} * 31.998 \text{ mg/mmol} * 25 \frac{\text{g dcw}}{\text{l}}}{\left(7.2 \frac{\text{mg}}{\text{l}} - 7.2 \frac{\text{mg}}{\text{l}} * 0.3\right)} = 1349/\text{h} = 22.49/\text{min} \quad (10)$$

The resulting k_{La} value of 22.49 /min is unusually high and represents a highly optimized process at the very peak of the growth of the culture. More typical k_{La} values found in literature range from around 1-15 /min, with values higher in the range being less common. (Doran, Morrissey & Carlson 2025a; Liu 2020) Therefore, further calculations will be made with a representative k_{La} value of 5 /min.

The gas flows needed to reach the needed k_{La} value for *E. coli*, as well as for CHO cells, are shown in Table 6.7. Note that the gas flow rates needed are outside of the experimentally investigated range.

Table 6.7. Shows the relation between gas flow and k_{La} for different aeration methods. The needed k_{La} and the consequently needed gas flow are shown for CHO cells and *E. coli*.

Aeration method	Linear regression	CHO		<i>E. coli</i>	
		k_{La} (/min)	Q_g (m ³ /h)	k_{La} (/min)	Q_g (m ³ /h)
Venturi valve	$k_{La} = 0.2858 * Q_g$	0.039	0.137	5	17.39
Pipe on pipe no agitation	$k_{La} = 0.0523 * Q_g$	0.039	0.747	5	95.6
Pipe on pipe 30% agitation	$k_{La} = 0.0452 * Q_g$	0.039	0.864	5	110.6
Sintered stone	$k_{La} = 0.0429 * Q_g$	0.039	0.910	5	116.6

6.4.2 Limits by maximum shear forces

To create the operational window, the limits for when the shear forces become lethal have to be found. This differs widely between different cell cultures and conclusions will therefore be drawn for CHO cells and *E. coli* separately.

As mentioned before, agitation generally creates higher shear conditions than aeration. Since the maximum impeller tip speed for CHO cells occurs at a lower agitation rate than the maximum power density, comparing Figure 6.8 and Figure 6.9, the impeller tip speed will be the limiting factor when determining the maximum agitation for a CHO cell culture. This means that the highest possible agitation for CHO cells, according to the calculations, is 1.19/s (72 rpm).

According to Figure 6.10, the maximum gas entrance velocity for CHO cells is reached at approximately 38 m³/h for venturi valve, 106 m³/h for pipe on pipe method and practically unlimited for sintered stone for normal bioreactor use. The maximum bubble size to not create lethal dissipation energy for CHO cells is about 0.33 cm according to the model used, as shown in Figure 6.11. Using the fact that bubbles are 10 times larger than the hole size, the sparging hole has to be at least 3 mm to not create too small bubbles, which create lethal dissipation energy. This indicates that sintered stone is not a viable option for CHO cells under the evaluated conditions.

As stated in Table 4.2, *E. coli* is generally shear resistant. Therefore, it will be assumed that in common operation conditions, the created shear forces will not be large enough to be lethal to the cell culture. Therefore, the limits of high agitation, bubble size and gas entrance velocity are assumed not to be limited by the cell culture under typical operating conditions and can be defined by what is optimal for aeration.

6.4.3 Final operational window

Using all results from the previous sections, resulting operational windows for aeration and agitation for each aeration method are shown in Figure 6.12, which combines the experimentally determined oxygen transfer with the calculated shear limits.

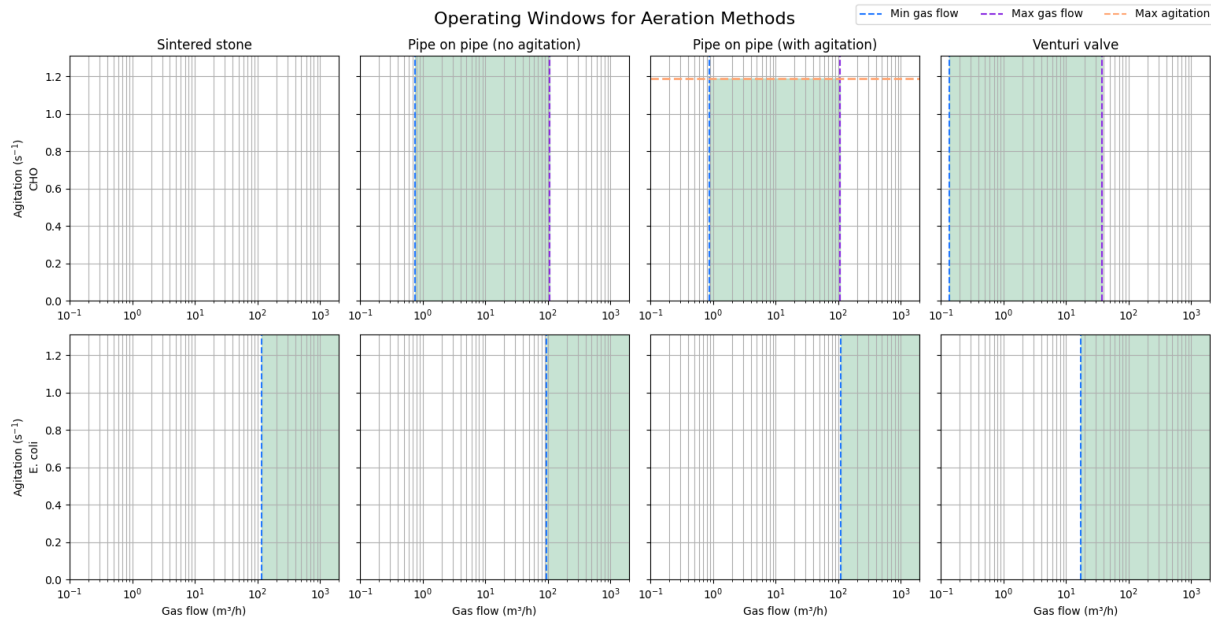


Figure 6.12. Operational window for CHO cells and *E. coli* for four aeration conditions (sintered stone, pipe on pipe without and with agitation, and venturi valve). The green shaded area indicates feasible operating conditions, and the dashed lines are the limiting conditions on maximum agitation (orange), minimum aeration (blue), and maximum aeration (purple).

7 Discussion

7.1 Aeration and the dynamic method

In the results from the experimental trials summarized in Figure 6.6, it is evident that higher gas flows result in higher k_La . Since k_La depends highly on the system setup, reactor design and medium, it is difficult to compare with values found in literature, but they are within the same order of magnitude as reported values. (Stanbury, Whitaker & Hall 2017b; Doran, Morrissey & Carlson 2025a).

7.1.1 Comparison of different aeration methods

As shown in Figure 6.6, the venturi valve achieved high k_La values at low gas flows, which is further illustrated in Figure 6.7 where the differences in oxygen transfer efficiency are clearly observed. This is likely due to the local zone of lower pressure breaking up the bubbles and high turbulence enhancing the gas-liquid mass transfer by the venturi valve. Additionally, the small bubbles have a high gas hold-up time in the reactor, further increasing the oxygen transfer.

Figure 6.6 and further Figure 6.7 show that the difference in k_La between pipe on pipe with and without agitation is relatively small. This is likely because the turbulence in the reactor was primarily from the aeration rather than the agitation. A trial at 50% agitation was therefore conducted, as seen in Figure 6.6, which shows only a slight increase in k_La . Although this caused gas hold-up around the shaft. For the agitation to have a higher impact on k_La , the agitation rate would have to be increased, or a different impeller design would have to be used. The marine impeller used in the trials is known to create low shear forces, meaning it is also less efficient at dispersing gas in comparison to, for example, a Rushton impeller. However, changing to a Rushton impeller would only be suitable for *E. coli* and not CHO cells, since only *E. coli* requires higher aeration efficiency and can handle high shear stress. (Doran, Morrissey & Carlson 2025b)

The difference between the two pipe on pipe aeration methods does not differ much from aeration with the sintered stone. As the pore size of the sintered stone is much smaller than the holes in the pipe on pipe, it generates smaller bubbles and thus a larger surface area of gas-liquid interfacial area. Additionally, larger bubbles from pipe on pipe have a short gas hold-up time in the reactor. The very small pores in the sintered stone might create a higher pressure drop, possibly reducing the gas throughput, although this cannot be certain in the conducted studies.

7.1.2 Evaluation of the dynamic method

Even if the dynamic method is an established and widely used method to determine the k_La value for a process, its accuracy may be limited under certain conditions. An assumption that it makes is that the media, or in this case water, in the entire system is homogenous. Whether this assumption holds true is not known and would require validation, for example by using multiple DO sensors at various positions in the reactor. With water being a low viscosity liquid and the sensor placement near the wall of the reactor, it may lead to a slight underestimation of the average DO concentration.

Before all processes, the DO sensor was calibrated in conditions similar to those it would be operated in, i.e., water at room temperature sparged by air. If this calibration is inaccurate, the accuracy of all results will be affected, although relative differences between conditions might

still be somewhat valid. Another important aspect of the dynamic method is that for the trials where the time from degassed water to saturation was very short, 12 minutes for the fastest sparging, the sensor's response time becomes a limiting factor. The sensor used (Mettler Toledo, InPro 6850i) has a response time of less than 90 s to reach 98% of the final value. When DO increases rapidly, the sensor reading lags behind, affecting the accuracy of the measured values.

7.1.3 Regression of k_La and gas flow

To create a reliable regression between k_La and gas flow rate, multiple trials for each sparging method across a range of values are needed. Since only four gas flows for all sparging methods apart from venturi valve method for which only two gas flows were investigated, a regression has a limited dataset to be based on. Moreover, the relationship between k_La and gas flow rate might be more complex than assumed in this report. Some studies suggest a linear fit, as is used in this report, while others show that logarithmic regression gives a better approximation. (Stanbury, Whitaker & Hall 2017b; Doran, Morrissey & Carlson 2025a) Since the aeration methods in this article differ widely from each other, it might not be that the same type of regression is the best fit for all. Figure 6.6 shows the regressions and their coefficient of determination. For the pipe on pipe method, it appears to be a good fit, whereas for the sintered stone, a logarithmic fit might be better, since a linear fit shows a lower R^2 value. Since the estimations on required aeration for *E. coli* and CHO cells in Table 6.7 are based on these regressions, any model inaccuracies are propagated into the calculated gas flow requirements. Since the required aeration for *E. coli* falls outside of the experimentally investigated range of gas flow rates, the extrapolation is a major source of uncertainty. This might be one of the main factors of error in Table 6.7 showing the needed gas flow for cultivation of especially *E. coli*.

7.2 Shear forces

The shear forces calculated are only estimations and need validation to confirm their accuracy. Ideally, a complex CFD model is needed to define the shear forces from aeration, agitation, and other sources, and how they interact.

7.2.1 Operational limits set by shear forces

The limiting factor on agitation for CHO cells was determined to be the tip speed with a detrimental level at 1.2 /s, which is of the same magnitude as other reported values. (Muralidharan, Bolduc & Davis 2024). This is also similar to the 30% agitation (1.5 /s) at which the agitation trials were conducted. Upper limits for gas flow for pipe on pipe estimated to 106 m³/h and for venturi valve to 38 m³/h based on the GEV constraints. These values are higher, yet of the same order of magnitude as 12 m³/h reported for CHO cells in another bioreactor setup. (Muralidharan, Bolduc & Davis 2024)

For aeration in the setup used, it was estimated that the small bubble size from the sintered stone generates high dissipation energy too high for the CHO cells. Estimating the impact on the cells by the dissipation energy from bubbles rupturing at the surface of the liquid is hard to determine. The model used in Equation 8 is based on a different bioreactor setup and may therefore not be accurately applicable. To improve the accuracy, a model specific to the bioreactor configuration would be needed. The model could also be made more accurate by including a model of the average and/or smallest bubble size created by a certain sparging method. Since the estimation that the bubble diameter is 10 times the hole size is used for the calculations represents a simplified assumption, the true minimum hole size might differ significantly from the 3.3 mm found in Figure 6.11.

7.2.2 Sub-lethal effects on cells

Throughout all calculations on shear forces, shear limits were defined based on lethal levels, however, shear forces might affect cells in more subtle ways, including cell metabolism and growth. The impact of lower shear conditions is specific for each process and would require further investigations. For example, cell stress might reduce growth rate, leading to lower oxygen demand. Here, no upper limit on shear stress was defined for *E. coli* under typical operating conditions, but when reaching high shear conditions, cell damage is expected.

7.2.3 Shear forces by the venturi valve

In this report, shear forces from the venturi valve were simplified to only include gas entrance. However, the process is more accurately described as entrainment rather than injection, which could better be estimated with another model. Moreover, the pressure drop in combination with the turbulent interactions between gas and liquid at the inlet may create high local shear conditions that could be detrimental to CHO cells. Experimental data on whether CHO cells can handle aeration through venturi valves, or similar designs, is limited, but based on the sensitivity of CHO cells, it can be expected that the venturi valve creates too harsh shear conditions. An alternative application for the venturi valve could be to saturate cell free medium in fed-batch operations. Further investigations are also required to determine whether the venturi valve generates too high shear stress even *E. coli*, which is of high interest since it is the most efficient aeration method according to the results of this report.

7.3 Optimal aeration configuration

To identify a preferred aeration method for cultivation of *E. coli* and CHO cells, it has to meet the oxygen demand while avoiding detrimental shear conditions. Ideally, high efficiency is also desirable to facilitate scale up to industrial size.

7.3.1 Optimal aeration configuration for CHO cells

Based on the experimental results, sintered stone is not a suitable option for CHO cells under the tested conditions due to the small bubbles generating too high energy dissipation. As discussed, there is a substantial risk of the venturi valve creating a harsh shear environment. Therefore, pipe on pipe aeration with gas flows of 0.8 m³/h, combined with a maximum agitation of 1.19 /s appears to provide the most suitable operating conditions for CHO cells.

7.3.2 Optimal aeration configuration for *E. coli*

For *E. coli*, aeration efficiency is of high importance to meet the high oxygen demand. As shear forces are assumed not to limit the options, venturi valve can be selected since it shows the most efficient aeration, facilitating scalability. An aeration of 18 m³/h using the venturi valve is therefore recommended. Although further investigations are required to confirm that shear forces associated with the venturi valve are not detrimental.

8 Conclusion

k_{La} was experimentally determined for the four aeration methods, with gas flows ranging from 0.5 m³/h to 5 m³/h. Based on the results, a mathematical relationship between k_{La} and gas flow rate was established and compared to the oxygen requirements of *E. coli* and CHO cells. This gave the lower gas flow limit for each aeration method, which was significantly higher for *E. coli* than for CHO cells.

Shear forces generated by aeration were estimated based on gas entrance velocity and the rupture of bubbles. The gas entrance velocity set the upper limit on gas flow for CHO cells, and the rupture of bubbles indicated that the sintered stone was not a viable option for aeration due to the energy dissipation from the small bubbles. As one of the aeration methods included agitation, the shear forces from agitation were found for CHO cells. *E. coli* was found to be sufficiently robust to tolerate the shear forces from aeration and agitation within typical operating conditions. The findings were presented as operational windows defining a feasible range for each aeration method.

Venturi valve is the recommended aeration method for *E. coli* due to being the most efficient. Minimum gas flow required to meet oxygen requirement at high cell density is approximated to 18 m³/h. For CHO cells, the recommended aeration method is pipe on pipe, with a maximum agitation of 1.2/s. The gas flow is recommended at 0.8 m³/h to meet its oxygen requirement.

This work shows how aeration and shear conditions are interdependent and can, in combination, define an operational window, guiding the selection of process parameters for precision fermentation and cultivated meat.

9 Future work

9.1.1 Validation of results

As no experimental trials were conducted with biological systems, the operational windows must be confirmed. Trials on whether the needed gas flow rates to meet the oxygen requirements for each aeration method must be conducted, as well as trials on maximum shear forces for aeration and agitation. As the equipment used is of pilot scale, experimental trials in industrial scale reactors must be made to validate their scalability.

9.1.2 Water compared to cell media

All experimental trials in this report were conducted using water, which differs in certain aspects to cell media. The differences are both in regard to the content of the media, usually being mainly sugars, salts and other nutrients, and the cells present. Oxygen solubility in medium is often 5-25% lower than in water, meaning that both the oxygen concentration at saturation and the saturation rate would differ if the trials had been made with medium. Higher viscosity by either the medium or a high cell density creates a resistance in oxygen transfer and reduces turbulence, meaning that k_{La} would decrease. (Doran, Morrissey & Carlson 2025a). If trials with mediums were conducted at the highest and lowest gas flows for all sparging methods, it would allow a reliable extrapolation from water to biological systems.

9.1.3 Operational window with more dimensions

The operational windows presented in this report are simplified representations of real process conditions. Additional factors, such as carbon dioxide accumulation or spatial gradients due to insufficient circulation, should also be considered. (Stanbury, Whitaker & Hall 2017b) The cost and environmental impact of operation are only considered as the oxygen transfer efficiency. Therefore, it is of interest for future investigations to consider these factors to develop more comprehensive operational windows, including more parameters.

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