Transport processes in currently used bioreactors

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von Herrn Christian Philipp Sieblist
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Gutachter:

Prof. Dr. rer. nat. habil. Andreas Lübbert

Prof. Dr.-Ing. Jochen Büchs

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CONTENTS

SUMMARY

Biopharmaceuticals roughly worth 5.2 billion euros were sold in Germany in 2010[1] and over 460 new biological active substances are in clinical development worldwide currently. However the particular production processes are of a high technical complexity and have to comply with legal requirements. These production processes are usually limited to few production sites and plants to minimize the risks and costs.

For the production of recombinant therapeutic proteins are mostly animal cell lines used, because they are able to perform complex glycosylation and posttranslational modifications. Only through these modifications gets a protein its therapeutic efficacy. For the cultivation of cell lines are bioreactors used. They range from microliter scale to production scale (*1 m³* to *25 m³*). Unlike bacteria and yeast possess mammalian cells no stabilizing cell walls. That is why they react very sensitively to alterations of their physical environment.

One important requirement for a production process is the physical characterization of production equipment in order to guarantee a robust procedure. Therefore detailed information of the fluid-dynamic operating parameters is necessary. Only with this knowledge a gentle cultivation at large scale with a high product yield can be guaranteed. The primary objective for the fluid dynamic process characterization and optimization is not the minimization of process costs but the compliance with manufacturing and production requirements defined by the regulatory authorities (*EMA / FDA*).

One of the most important tasks of a stirred bioreactor is to ensure and maintain the homogeneity of the culture media. In this thesis it was shown that mixing processes in stirred vessels can be modeled by using *CFD*. The models were validated using experimental data. Furthermore it was shown that the mixing process unlike mostly shown in literature is not solely dependent on physical power input. The shape and arrangement of the stirring elements as well as the position of the place of addition of reactants or correction substances have shown a significant influence on the time flow of the mixing process.

The characterization of mass transfer from the gas phase into the liquid phase in a stirred vessel is more difficult to perform. For a first approximation of k_La values the total power input can be used. Especially the local energy input plays an important role at the gas dispersion which was verified using experimental data. A *30%* higher k_La-value could be determined for the one stage impeller system in comparison to the three stage systems by equal global energy inputs. To describe the mass transfer under cell culture conditions the well-known correlation published by van't Riet (1997) can be used. However, the by van't Riet propagated borders of the correlation constants are not valid for this special case.

The dispersion of gas bubbles in the liquid does not only influence the mass transfer of oxygen into the cultivation medium it also influences the power transmission from the stirrer to the liquid. In gen-

[1] von Holleben, M., Pani, M. Heinemann, A.M., Medizinische Biotechnologie in Deutschland 2011, Biopharmazeutika: Wirtschaftsdaten und Nutzen der Personalisierten Medizin, *The Boston Consulting Group GmbH, München,* **2011**

eral this effect is neglected in literature about cell culture reactors. By the means of a *10 m³* bioreactor equipped with a three stage impeller system and operated under cell culture conditions was shown that power losses in the range of *50%* are possible. These losses are caused by the reduced specific density of the liquid as well as the formation of so-called "gas cavities" after the impeller blades.

The development of such gas cavities was visualized for Rusthon turbines as well as for hollow-blade impellers. It was done with a newly developed video recording technology co-rotating with the agitator shaft. In comparison with the experimental data of cavity formations after impeller blades the *CFD*-calculated data was a good match. Especially dominant was the growth of gas cavities after so-called "low-shear elephant ear impellers". These are currently often recommended for cultivation of animal cells. The use of such agitators must be seen critically especially for large scale bioreactors because of the formation and rupture process of the cavities which lead to mechanical instabilities. These instabilities can be measured as torque fluctuations and be perceived as vibrations at the reactor.

As well-known from literature the effective removal of CO_2 formed by cell respiration is one of the major problems in large production bioreactors. As shown by measurement results the dwell time of the gas bubbles in the medium plays an important role for the mass transport process of CO_2. Starting at a scale of *300 L* the determined k_La-values of CO_2, unlike the k_La-values of O_2, cannot be affected by a change of the specific interfacial area. An increase in CO_2 stripping can only be reached by an increase of the aeration rate in large reactors. To describe the mass transfer of CO_2 and O_2 a mathematical model is presented in this thesis. For the model the gas residence time was taken into account.

Another challenge to master is the sensitivity of animal cells against shear stress. One of the principally used substances as protection against shear stress is Pluronic® F68. In commercial cell culture media it is used in concentrations between *1* and *3 g/L*. In this thesis it was shown that the mass transfer via the specific interfacial area is reduced by *50%* in addition of Pluronic® F68 concentrations well below the critical micelle concentration. For a description of the obtained effects the penetration theory by Higbie (1935) and the surface renewal theory by Dankwerts (1951) were used. It was detected that the repression of bubbles movement is the main cause for the strong decrease in mass transfer.

For Pluronic® F68 concentrations above *0.1 g/L* it was observed that it comes to changes in bubble appearance and sizes. But these changes strongly depend on the used sparger type. By using the measured bubble sizes it was discovered that the change on mass transfer coefficient (k_L) is very low above the critical micelle concentration. Further changes on overall mass transfer coefficient at higher Pluronic® F68 concentrations are mainly based on increasing specific mass transfer area (a) and partly on increasing gas holdup (ε_G).

As shown with the Pluronic® F68 investigation plays the bubble size an important role respective the mass transfer of oxygen. One of the non-characterized sparger types in literature is the sintered sparger. Due to the production process is the material quality subject to fluctuations. With regard to the degree of porosity and the pore size variations up to *40%* are possible. It was necessary to develop a non-destructive method for the characterization of the sintered spargers in production bioreactors. Therefore a testing procedure is presented which use the oxygen mass transfer coefficient (k_La) as an evaluation criterion. Performing the test on sinter spargers of one production lot showed a variation up to *40%*. In order to realize an efficient testing in a production environment an automated test facility was constructed. This test rig is able to test sintered spargers by using various gas throughputs. The results are checked against a reference sparger.

The content presented in this cumulative doctoral dissertation was published in peer reviewed journals and books as following five articles: [1-5]

1. Sieblist, C.; Jenzsch, M.; Pohlscheidt, M.; Lübbert, A., 2.06 - Bioreactor Fluid Dynamics. In: *Comprehensive Biotechnology (Second Edition)*, Moo-Young, M., (Ed.) Academic Press: Burlington, **2011**; pp 47-62.

2. Sieblist, C.; Jenzsch, M.; Pohlscheidt, M.; Lübbert, A., Insights into large-scale cell-culture reactors: I. Liquid mixing and oxygen supply, *Biotechnology Journal*, **2011,** 6, (12), 1532-1546.

3. Sieblist, C.; Hägeholz, O.; Aehle, M.; Jenzsch, M.; Pohlscheidt, M.; Lübbert, A., Insights into large-scale cell-culture reactors: II. Gas-phase mixing and CO_2 stripping, *Biotechnology Journal*, **2011,** 6, (12), 1547-1556.

4. Sieblist, C.; Jenzsch, M.; Pohlscheidt, M., Influence of Pluronic® F68 on mass transfer under cell culture process conditions, *Biotechnology Progress*, **2012,** submitted.

5. Sieblist, C.; Aehle, M.; Pohlscheidt, M.; Jenzsch, M.; Lübbert, A., A test facility for fritted spargers of production-scale-bioreactors, *Cytotechnology*, **2010,** 63, (1), 49-55.

ZUSAMMENFASSUNG

Im Jahr 2010 wurden in Deutschland Biopharmazeutika im Wert von ca. 5,2 Milliarden Euro verkauft[2] und derzeit befinden sich weltweit mindestens 460 neue biologische Wirkstoffe in der klinischen Entwicklung. Die jeweiligen Herstellprozesse sind jedoch technisch sehr aufwendig und gesetzlich mit hohen Anforderungen belegt. Zur Minimierung von Risiken und Aufwand ist die Herstellung daher meist auf wenige Produktionsstandorte und Anlagen begrenzt.

Derzeit werden meist tierische Zelllinien zur Produktion rekombinanter therapeutischer Proteine eingesetzt, da diese die komplexen Glykosilierungen und posttranslationalen Modifikationen korrekt durchführen können. Erst mittels dieser Modifikationen erhalten die Proteine ihre therapeutische Wirksamkeit. Für die Kultivierung dieser Zelllinien kommen Bioreaktoren vom Mikrolitermaßstab bis hin zu Rührkesselbioreaktoren mit Größenordnungen von *1m³* bis *25m³* zum Einsatz. Im Gegensatz zu Bakterien und Hefen besitzen jedoch Säugerzellen keine stabilisierende Zellwand. Sie reagieren daher sehr empfindlich auf Veränderung von physikalischen Einflüssen.

Eine wichtige Anforderung an einen solchen Produktionsprozess ist, dass dieser technologisch charakterisiert ist, um eine robuste Fahrweise zu gewährleisten. Dafür ist eine genaue Kenntnis der fluiddynamischen Betriebsparameter notwendig. Durch sie kann eine schonende Kultivierung im großen Maßstab bei gleichzeitig hoher Produktausbeute gewährleistet werden. Vorrangiges Ziel bei der fluiddynamischen Prozesscharakterisierung und -optimierung ist jedoch nicht die Minimierung der Prozesskosten, sondern die Einhaltung der durch die Arzneimittelbehörden (*EMA / FDA*) vorgegebenen Herstellungs- und Produktionsanforderungen.

Eine der wichtigsten Aufgaben eines Rührkesselbioreaktors ist die Gewährleistung und Aufrechterhaltung der Homogenität im Kulturmedium. In dieser Arbeit konnte gezeigt werden, dass Mischprozesse in Rührkesselbioreaktoren sehr gut mittels *CFD* modelliert und mittels experimenteller Daten validiert werden können. Weiterhin konnte gezeigt werden, dass der Durchmischungsvorgang, anders als zumeist in der Literatur dargestellt, nicht alleine vom physikalischen Leistungseintrag abhängig ist. Die Form und Anordnung der Rührorgane, sowie die Position des Zugabeortes von Korrekturmitteln oder Reaktanten zeigen einen signifikanten Einfluss auf den zeitlichen Verlauf des Durchmischungsvorganges.

Weitaus schwieriger ist die Charakterisierung des Stoffübergangs von der Gas- in die Flüssigphase in einem Rührkessel. Für eine erste Approximation von k_La-Werten kann der totale Leistungseintrag verwendet werden. Wie jedoch mittels experimenteller Daten gezeigt werden konnte, spielt bei der Gasdispergierung besonders der lokale Energieeintrag eine Rolle. Bei gleichen globalen Energieeinträgen konnten bei einstufigen Rührsystemen im Mittel um *30%* höhere k_La-Werte ermittelt werden als bei dreistufigen Systemen. Zur Beschreibung des Stoffüberganges unter Zellkulturprozessbedingungen kann gut die von van't Riet (1997) veröffentlichte Korrelationsfunktion eingesetzt werden.

[2] von Holleben, M., Pani, M. Heinemann, A.M., Medizinische Biotechnologie in Deutschland 2011, Biopharmazeutika: Wirtschaftsdaten und Nutzen der Personalisierten Medizin, *The Boston Consulting Group GmbH, München*, **2011**

Jedoch können hier nicht mehr die von van't Riet propagierten Grenzen der Korrelationskonstanten eingehalten werden.

Die Dispergierung von Gasblasen in der Flüssigkeit beeinflusst nicht nur den Stoffübergang von Sauerstoff in das Kultivierungsmedium, sondern auch die Leistungsübertragung vom Rührorgan in die Flüssigkeit. Dieser Effekt wird in der Literatur für Zellkulturreaktoren meist vernachlässigt. So konnte am Beispiel eines dreistufigen Zellkulturreaktors mit *10m³* Arbeitsvolumen gezeigt werden, das unter Zellkulturbedingungen Leistungsverluste von bis zu *50%* auftreten können. Dies wird durch die verringerte relative Dichte als auch durch die Ausbildung von sogenannten „Gas-Cavities" an den Rührerblättern verursacht.

Mit einer mit der Rührwelle mitrotierenden Videoaufnahmetechnik konnte die Entstehung der Gas-Cavities sowohl bei Scheiben- als auch bei Hohlblattrührern gezeigt werden. Der visuelle Vergleich von mittels *CFD* berechneten Turbulenzschleppen an Rührorganen zeigt eine sehr gute Übereinstimmung mit den für diese Arbeit experimentell ermittelten Cavity-Formationen. Besonders interessant ist auch das Aufwachsen von Gas Cavities hinter sogenannten „scherarmen Elephant Ear Impellern" zu sehen, welche derzeit häufig für die Kultivierung von tierischen Zellen propagiert werden. Der Einsatz von solchen Rührern ist besonders bei großen Bioreaktoren kritisch zu sehen, da der Bildungs- und Abrissprozess der Cavities zu mechanischen Instabilitäten führt. Diese lassen sich deutlich als Drehmomentschwankungen als auch als Vibrationen am Reaktor wahrnehmen lassen.

Wie aus der Literatur bekannt ist, ist der effektive Abtransport des von den Zellen bei der Atmung gebildeten CO_2 eines der Hauptprobleme bei großen Produktionsbioreaktoren. Wie mittels Messergebnissen gezeigt werden konnte, spielt für den Stofftransport von CO_2 die Verweilzeit der Gasblasen im Medium eine besondere Rolle. Schon ab einem Maßstab von *300 L* kann der ermittelte k_La-Wert von CO_2, anders als der von O_2, nicht mehr durch eine Veränderung der spezifischen Phasengrenzfläche beeinflusst werden. Eine Steigerung des CO_2-Strippings ist bei großen Reaktoren daher nur mittels der Erhöhung der Begasungsrate zu erreichen. Zur Beschreibung des Stoffübergangs von O_2 und CO_2 wird in dieser Arbeit ein mathematisches Modell für den Stofftransport unter Einbeziehung einer mittleren Gasverweilzeit beschrieben.

Ein weiteres Problem bei der Kultivierung von tierischen Zellen ist die Empfindlichkeit der Zellen gegenüber Scherstress. In der kommerziellen Zellkulturtechnik wird zum Schutz der Zellen vor Scherstress meist Pluronic® F68 in Konzentrationen von *1* bis *3 g/L* eingesetzt. In dieser Arbeit wurde anhand von Untersuchungen gezeigt, dass der Stoffübergang an der Phasengrenzfläche schon bei Zusatz von Pluronic® F68 Konzentrationen weit unter der kritischen Mizellkonzentration um rund *50%* reduziert wird. Zur Beschreibung der ermittelten Effekte wurde die Penetrationstheorie nach Higbie (1935) und die Oberflächenerneuerungstheorie nach Dankwerts (1951) verwendet. Es konnte dabei demonstriert werden, dass die Unterdrückung der Bewegung der Blasenoberflächen die hauptsächliche Ursache für den starken Abfall der ermittelten k_La-Werte ist.

Zusätzlich konnte bei Konzentration oberhalb *0.1 g/L* Pluronic® F68 Veränderungen der Blasengrößen als auch der Blasenform beobachtet werden. Diese Veränderungen hängen stark vom eingesetzten Begasertyp ab. Unter Verwendung der gemessenen Blasengrößen konnte gezeigt werden, dass die Veränderungen des Stofftransferkoeffizienten (k_L) oberhalb der kritischen Mizellkonzentration gering sind. Veränderungen des volumenspezifischen Stofftransferkoeffizienten bei höheren Pluronic® F68-

Konzentrationen beruhen hauptsächlich auf der Erhöhung der spezifischen Stofftransferfläche und teilweise auf der Erhöhung des Gas-Holdup.

Wie durch die Pluronic® F68-Untersuchung gezeigt, spielt die Blasengröße für den Stofftransfer von Sauerstoff eine wichtige Rolle. Ein bis jetzt in der Literatur nicht genauer charakterisierter Begasertyp ist der Frittenbegaser. Schon durch den eigentlichen Herstellungsprozess bedingt kommt es im Sintermaterial zu Schwankungen im Porositätsgrad bis *40%* und zu unterschiedlichen Porengrößenverteilungen. Zur zerstörungsfreien Charakterisierung von in Produktionsreaktoren eingesetzten Sinterfritten, war die Entwicklung eines entsprechenden Testungs- und Validierungsverfahrens notwendig. Bei dem in dieser Arbeit vorgestellten Verfahren wurde der Sauerstofftransferkoeffizient (k_La) als Bewertungskriterium eingesetzt. Bei den durchgeführten Untersuchungen an Begaserfritten aus einem Produktionslot konnten Unterschiede von bis zu *40%* ermittelt werden. Um eine effiziente Testung im Produktionsumfeld zu gewährleisten, wurde eine Teststation entwickelt, mit welcher die entsprechenden Begasungselemente bei unterschiedlichen Begasungsraten im Vergleich zu einem Referenzelement vollautomatisch getestet werden können.

Der Inhalt dieser kumulativen Dissertation wurde in „peer-reviewed"-Zeitschriften und Büchern als folgende fünf Artikel veröffentlicht: [1-5]

1. Sieblist, C.; Jenzsch, M.; Pohlscheidt, M.; Lübbert, A., 2.06 - Bioreactor Fluid Dynamics. In: *Comprehensive Biotechnology (Second Edition)*, Moo-Young, M., (Ed.) Academic Press: Burlington, **2011**; pp 47-62.

2. Sieblist, C.; Jenzsch, M.; Pohlscheidt, M.; Lübbert, A., Insights into large-scale cell-culture reactors: I. Liquid mixing and oxygen supply, *Biotechnology Journal*, **2011,** 6, (12), 1532-1546.

3. Sieblist, C.; Hägeholz, O.; Aehle, M.; Jenzsch, M.; Pohlscheidt, M.; Lübbert, A., Insights into large-scale cell-culture reactors: II. Gas-phase mixing and CO_2 stripping, *Biotechnology Journal*, **2011,** 6, (12), 1547-1556.

4. Sieblist, C.; Jenzsch, M.; Pohlscheidt, M., Influence of Pluronic® F68 on mass transfer under cell culture process conditions, *Biotechnology Progress*, **2012,** submitted.

5. Sieblist, C.; Aehle, M.; Pohlscheidt, M.; Jenzsch, M.; Lübbert, A., A test facility for fritted spargers of production-scale-bioreactors, *Cytotechnology*, **2010,** 63, (1), 49-55.

CHAPTER 1

BIOREACTOR FLUID DYNAMICS

ABSTRACT: Flows are induced in bioreactors in order to mix the culture and to support mass transfer. These two tasks are addressed for the most important types of bioreactors, aerated stirred tanks and bubble columns. Insights into the basic mechanisms are provided by discussing several basic experimental results, for instance mixing time and flow velocity measurements, which consider the gas as well as the liquid phase. The experimental data show that the fluid flow velocity patterns usually shown in textbooks do not consider the flow properties essential for mixing and mass transfer. Also, computational fluid dynamic results are compared with experimental results to demonstrate that many aspects of the two-phase gas–liquid flows in bioreactors are mechanistically understood.

KEYWORDS: Bioreactors; CFD applications; Flow patterns; Fluid mixing; Gas residence times; Mass transfer; Measurement techniques

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CHAPTER 2

INSIGHTS INTO LARGE SCALE CELL CULTURE REACTORS: I. LIQUID MIXING AND OXYGEN SUPPLY

ABSTRACT: In the pharmaceutical industry it is state of the art to produce recombinant proteins and antibodies with animal cell cultures using bioreactors with volumes of up to *20 m^3*. Recent guidelines and position papers for the industry by *FDA* and *EMA* stress the necessity of mechanistic insights into large scale bioreactors. A detailed mechanistic view of their practically relevant subsystems is required as well as their mutual interactions, i.e. mixing or homogenization of the culture broth and sufficient mass and heat transfer. In large scale bioreactors for animal cell cultures different agitation systems are employed.

Here we discuss details of the flows induced in stirred tank reactors relevant for animal cell cultures Also solutions of the governing fluid dynamic equations obtained with the so-called computational fluid dynamics (*CFD*) are given. Experimental data obtained with improved measurement techniques are shown. The results are compared to previous studies and it is found that they support current hypotheses or models. Progress in improving insights requires continuous interactions between more accurate measurements and physical models. The paper aims at promoting the basic mechanistic understanding of transport phenomena that are crucial for large-scale animal cell culture reactors.

KEYWORDS: cell culture, oxygen mass transfer, mixing, large scale bioreactors

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CHAPTER 3

INSIGHTS INTO LARGE SCALE CELL CULTURE REACTORS: II. GAS–PHASE MIXING AND CO_2 STRIPPING

ABSTRACT: Most discussions about stirred tank bioreactors for cell cultures focus on liquid-phase motions and neglect the importance of the gas phase for mixing, power input and especially CO_2 stripping. Particularly in large production reactors CO_2 removal from the culture is known to be a major problem. Here we show that stripping is majorly affected by the change of the gas composition during the movement of the gas-phase through the bioreactor from the sparger system towards the head space. A mathematical model for CO_2-stripping and O_2-mass transfer is presented taking gas residence times into account. The gas phase is not moving through the reactor in form of a plug flow as often assumed. The model is validated by measurement data. Further measurement results are presented that show how the gas is partly recirculated by the impellers thus increasing the gas residence time. The gas residence times can be measured easily with stimulus-response techniques. The results give further insights on the gas residence time distributions in stirred tank reactors.

KEYWORDS: carbon dioxide, cell culture, large-scale bioreactors, mass transfer, mixing

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CHAPTER 4

INFLUENCE OF PLURONIC® F68 ON MASS TRANSFER UNDER CELL CULTURE PROCESS CONDITIONS

ABSTRACT: Pluronic® F68 is one of the most used shear protecting additives in cell culture cultivations. It is well known from literature that such surface-active surfactants lower the surface tension at the gas-liquid interface, which influences the mass transfer. In this study, the effect of Pluronic® F68 on oxygen mass transfer in a aqueous solutions under cell culture process conditions was examined. Therefore the gassing in / gassing out method and bubble size measurements was used. At low concentrations of *0.02 g/L*, a *50%* reduction on mass transfer was observed for all tested spargers and working conditions. An explanation of the observed effects by means of Higbie's penetration or Dankwerts surface renewal theory was applied. It could be demonstrated that the suppressed movement of the bubble surface layer is the main cause for the significant drop down of the k_La-values.

For Pluronic® F68 concentrations above *0.1 g/L* it was observed that it comes to changes in bubble appearance and bubble size strongly dependent on the used sparger type. By using the bubble size measurement data it could be shown that only small changes in mass transfer coefficient (k_L) take place above the critical micelle concentration. Further changes on overall mass transfer at higher Pluronic® F68 concentrations are mainly based on increasing of gas holdup and, more important, by increasing of the surface area available for mass transfer.

KEYWORDS: Pluronic® F68, oxygen mass transfer, cell culture, large scale bioreactors

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1 INTRODUCTION

The demand of complex therapeutic proteins and antibodies for the detection and treatment of diseases such as cancer has increased significantly in the past few decades. Between 1950 and 2008 a total of 1222 new molecular entities were approved by the regulatory authorities, and recombinant proteins, antibodies and antibody drug conjugates will be the major source of revenues in future.[1, 2] Due to their ability to properly fold and glycosylate such proteins, animal cells are often the ideal expression system and the *Chinese Hamster Ovary (CHO)* cells have become the working horse of the industry[3-5].

The cultivation of mammalian cells requires complex media design, specific bioreactor design and robust process control systems[6]. These requirements are based on the specific characteristics of mammalian cells, like for example a missing cell wall, which makes them sensitive to shear force and fluctuation in the cell culture environment.[7, 8] Therefore, the bioreactor has to realize sufficient homogenization and mass transfer[9, 10] at low shear force.[11, 12] Hydrodynamic shear force is caused by several means in bioreactors. In most cases aeration by air sparging or mechanical power input by agitation of the culture to achieve homogeneous mixing and sufficient mass transfer (O_2 and CO_2) is the major source of shear force. Amongst others, these factors are a function of geometry and design of the bioreactor including the impeller, the sparger design affecting primary bubble size, power input and tip speed of the impeller, as well as aeration or sparge rates. Since in general low power inputs are used in animal cell cultures, the most dominating source of shear force is associated with aeration, in particular bubble burst at the liquid surface.[7, 13-17] Power input by impellers and rising of bubbles in the liquid is of secondary importance for shear force.[18-21]

Besides these physical factors, the media composition and the use of additives have a major influence on mass transfer, shear protection and fluid dynamic of the bioreactor. To minimize the impact of shear force to the cells a lot of research has been conducted to protect the cells by different media additives. Experiments were performed with Methylcellulose[7], starch solutions, protein containing solutions (Albumin, serum, etc.) or non-ionic tensides. Effective protection could be demonstrated by different serum and non-ionic tenside (e.g. Pluronic®) [22]. Serum of animal or human origin is like any other non-chemically defined media component not favorable in production processes due to contamination risks and significant batch-to-batch variability. Hence, Pluronic® and other non-ionic tensides are commonly used in the base media formulation of most manufacturing processes. The concentration can vary broadly in commercially available media – for example Pluronic® F68 varies in a range of *0.5-3 g/L*. [23-25]

The efficiency and effectiveness of Pluronic® F68 in relation to protection of cells against shear force has been described previously.[17, 18, 21, 26-33] An overview to Pluronic® polyols and cell protection is described in the Encyclopedia of Industrial Biotechnology[24]. However, most of these studies were looking on more global phenomena and shear protection of cell cultures. Fundamental answers to the theory of the mode of actions in terms of mass transfer influence have not been given. In addition, some of the conclusions described in literature are contrary regarding the effect of Pluronic® F68 on mass transfer. Some authors have reported a reduction[29, 34, 35] while others reported a significant increase of mass transfer in their studies[36, 37]. In several cases a mixture of media components, known to have an influence on surface tensions and viscosity, were tested and therefore the effect of Pluronic® F68 and other surface active components on mass transfer could not be differentiated.

This article will present new data focusing on the effect of Pluronic® F68 in media on mass transfer, bubble size distribution and physical effects in relation to the published mass transfer models by Lewis and Whitman[38], Higbie[39] and Danckwerts[40] to deliver a more comprehensive view on the effect of Pluronic® F68 on mass transfer.

2 MATERIALS AND METHODS

2.1 Stirred Tank Equipment

For the determination of the mass transfer coefficient k_La a *400 L* acrylic glass model bioreactor was used (T_D*=640 mm*, H/T_D *= 1.6*). The tank was fully baffled (w/T_D *= 0.1*) and equipped with a three stage stirrer system (D/T_D*= 0.33*, Power Number: *10.15*). The equipment and geometry was similar to the existing large scale production. The working volume of *330 L* is known to be sufficient to represent large scale behaviors[41]. A principle sketch of the used system can be seen in Figure 1.

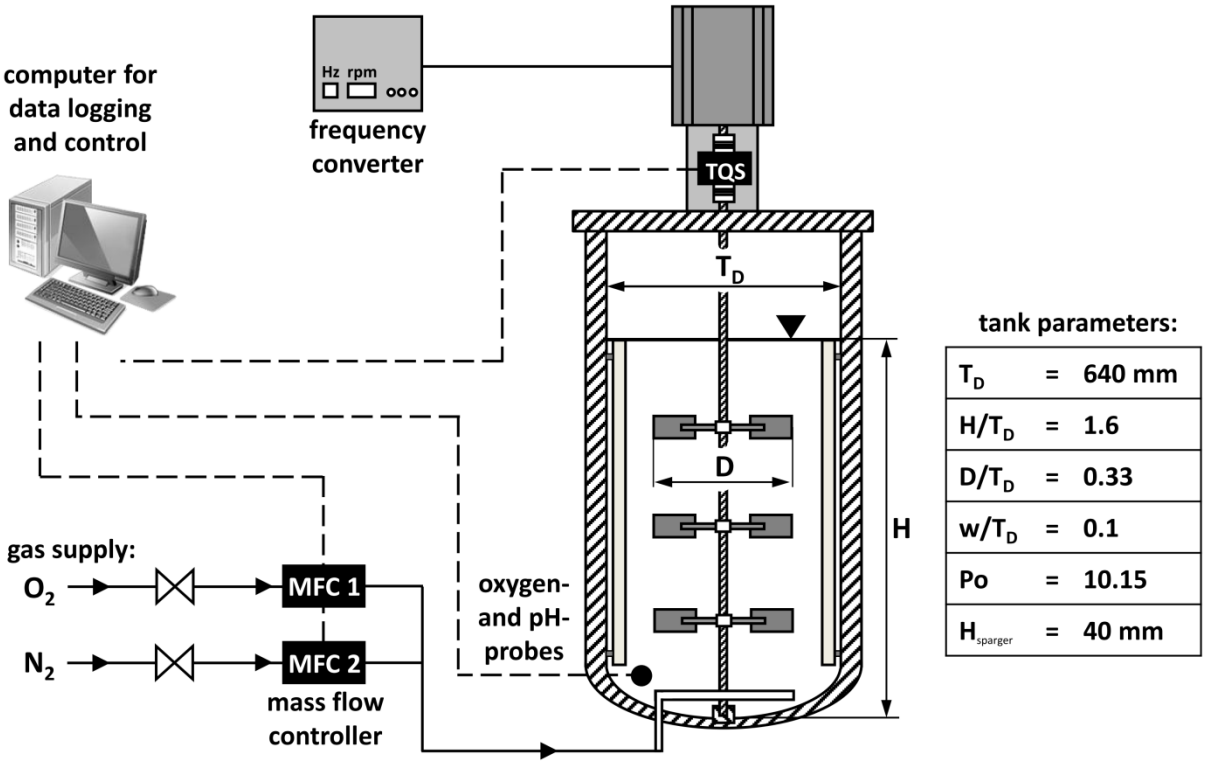

Figure 1. Principle sketch of the system.

The mechanical power input and the current stirrer speed was monitored by a torque sensor (DRFL-II, *20 Nm*, ETH-Messtechnik, Gschwend, Germany) which was installed between stirrer shaft and motor. To determine the power input, friction and bearing losses of the stirring system were considered by measurements in the unfilled tank. The effective torque was calculated according to equation 1:

$$M_{effective} = M_{loaded} - M_{empty} \quad (1)$$

and converted to the volume specific power input by equation 2:

$$\left(\frac{P}{V}\right) = \frac{2 \cdot \pi \cdot n \cdot M_{effective}}{V} \quad (2)$$

For the mass transfer experiments, four different aeration systems were tested. Figure 2 shows the different aeration systems.

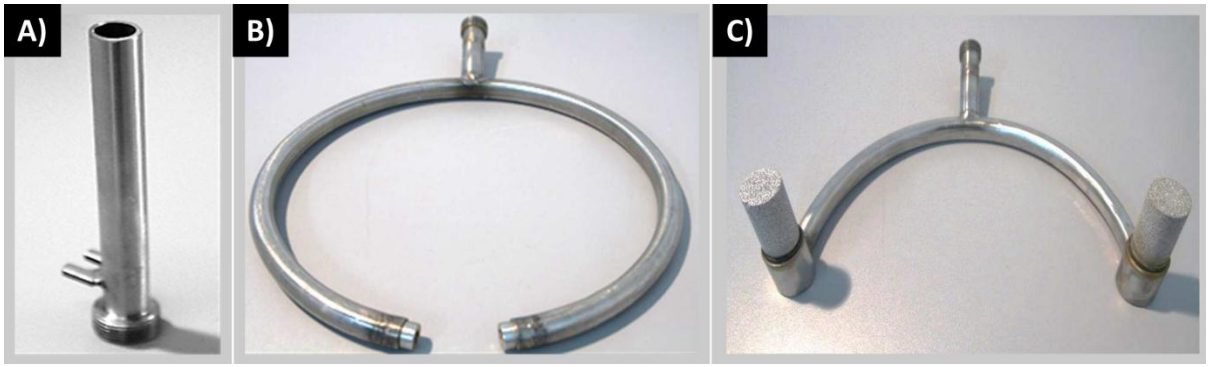

Figure 2. Used sparger systems: A) Open tube sparger [∅*10 mm*] B) Ring sparger [*18 holes, ∅0.5 mm*] C) Sinter sparger [two sinter elements with *47-100 µm* pore size or two sinter elements with *20-47 µm* pore size].

These sparger systems are representative for systems typically used in large scale cell culture production and were installed in the model reactor *40 mm* above the dished vessel bottom.

The open tube sparger (A) had a tube diameter of *10 mm* and was positioned so that the open pipe ranges up *10 mm* into the outer diameter of the stirrer. The ring sparger (B) was equipped with eighteen *0.5 mm* holes on top. The mean ring diameter was *200 mm*.

The third sparger was a sintered sparger system (C) which was equipped with two sintered sparging elements with an outer diameter of *22 mm* and a cylindrical height of *35 mm*. For the experiments, two types with different pore size ranges were available. The first type had pore size range of *47-100 µm*. The second tested sintered sparger had a pore size of *20-47 µm*. The porosity of both types was *40-60%*. The sinter sparger elements were installed to a ring with a mean diameter of *190 mm*.

The gas flow of air and nitrogen was realized by calibrated mass flow controllers manufactured by Vögtlin Instruments AG, Germany (red-y smart controller, GSC-B, *0-5 sLpm*, GSC-D, *0-30 sLmin*, ± *1.0%*).

2.2 Methods

2.2.1 Mass transfer measurements for O_2

The most important parameter to characterize and quantify the mass transfer is the volume-specific mass transfer coefficient (k_La) which describes the sorption velocity of a gas into a liquid medium. The experimental determination was performed with the dynamic gassing in/ gassing out method.[42, 43]

A saturation range of *20%* to *80%* oxygen as recommended by Liepe[44] was used during the experiments. Details of this method can be found elsewhere.[45, 46] The dissolved oxygen concentration was measured by a fluorescence sensor spot in the bottom of tank (PreSens - Precision Sensing GmbH, Germany) and converted by a data transmitter (PreSens Fibox 3, PreSens, Germany). As proposed by Linek[47] and van't Riet[48] the time constant of the measurement probe can be neglected if following condition is fulfilled:

$$t_{P63} << \frac{1}{5 \cdot k_L a} \tag{3}$$

Because the determined response time (t_{P63}) of the fluorescence probe was low (in this case *2 s*), a correction of the oxygen mass transfer coefficient was not necessary in all cases. The determination of the mass transfer coefficient was performed by:

$$OTR = \frac{dc_L}{dt} = -k_L a \cdot \left(c^* - c_L\right) \tag{4}$$

Where c^* is the maximum dissolved oxygen solubility and c_L is current measured concentration level of oxygen in the liquid. After integration, the mass transfer coefficient can be determined by:

$$k_L a = \frac{\ln\left(\frac{c^* - c_L}{c^* - c_{L,0}}\right)}{\Delta t} \tag{5}$$

All measurements were performed at $T=22 \pm 2°C$ and repeated three times. Since the data accuracy was within a range of *+/- 3%*, only the average is plotted on the graphs below.

2.2.2 Bubble Diameter Measurements

For the determination of the bubble diameter a Laser – light scattering system from Oxford Lasers Ltd. was used. The system measures the size and shape of bubbles by an image-based analysis. For recording of bubbles images, a high-speed *CCD* (charge coupled device) camera, a laser generator and a squared glass vessel (*300x300x900 mm*) was used, as shown in Figure 3.

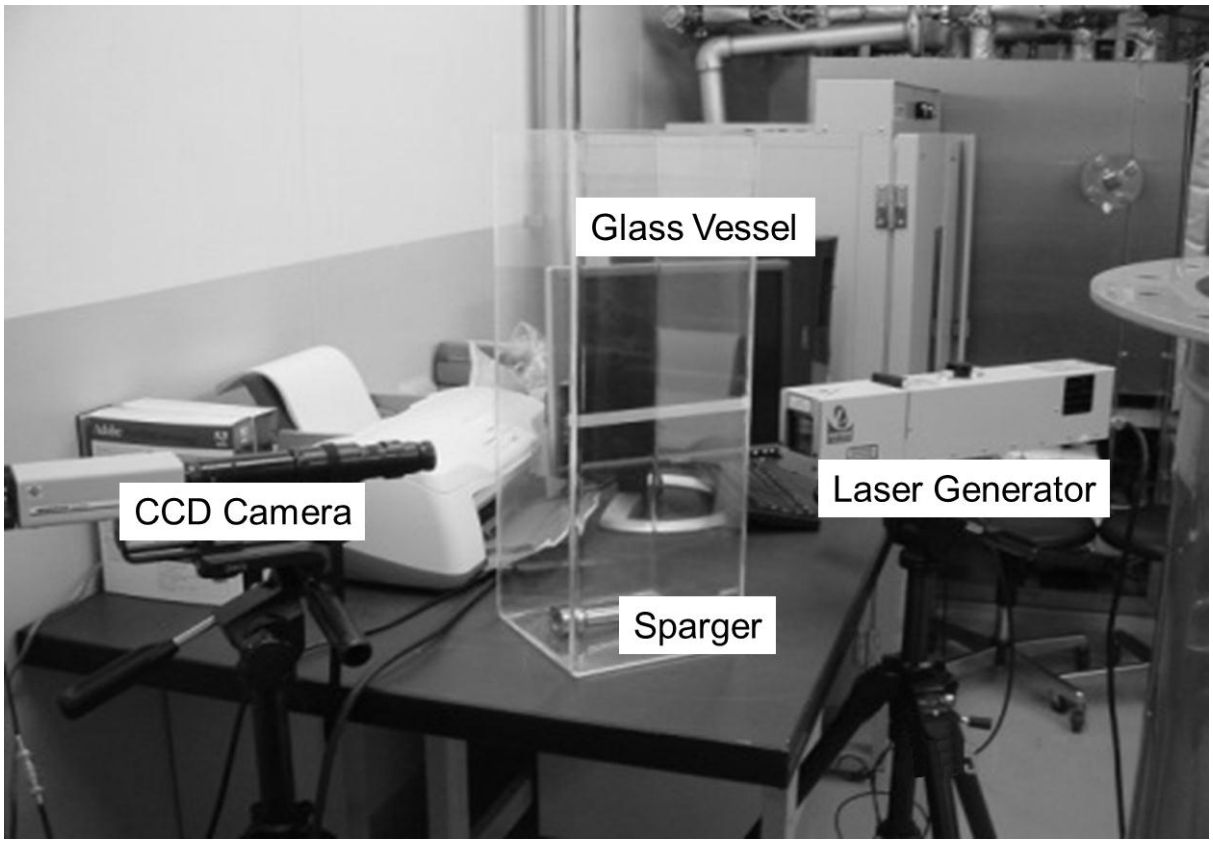

Figure 3. Used equipment for the bubble size measurements

The use of a rectangular vessel was necessary to prevent image deformations from curved walls. The bubble diameters of the different sparging rates and aeration systems were analyzed by specific software (VisiSize Solo, Oxford Lasers Ltd., UK). For comparison, the so-called Sauter diameter (d_{P32}) was determined[49]. It represents the mean bubble diameter, which has the same volume/surface area ratio as the entire gas flow[50]. It can be calculated by:

$$d_{P32} = \frac{\sum_{i=1}^{n} d_{B,i}^{3}}{\sum_{i=1}^{n} d_{B,i}^{2}} = \frac{6 \cdot \varepsilon_G}{a} \qquad (6)$$

Here $d_{B,i}$ represents the measured bubble size, ε_G the mean relative bubble fraction and a the specific mass transfer area. Typically, the Sauter diameter will be estimated from measured bubble size distribution and is the *32nd* percentile of cumulative size distribution.

2.3 Chemicals and Solutions

2.3.1 Media

In all cases, purified water with an addition of *8.7 g/L NaCl* was used as primary media to reach a comparable osmolality to typical cell culture media (*300 mOsm/kg)* and to prevent coalescence of the gas bubbles.[51-53] To this basal solution, different concentrations of Pluronic® F68 were added.

2.3.2 Pluronic® F68

Pluronic® is a trademark of block copolymer surfactants developed in the 1950s. It is lubricating, low-foam and or foam absorbing non-ionic surfactant made from polyethylene and polypropylene. Pluronic® belongs to the group of amphiphilic polymers which have hydrophilic (ethylene oxide) and hydrophobic (propylene oxide) polymer strands.

For these experiments Lutrol® F68 (INCI: Polaxamer 188, BASF Corporation, United States) was used. The brand Lutrol® F68 is the pharmaceutical grade quality of Pluronic® F68. Therefore, it will be referred to as Pluronic® F68 or PF68 henceforth in this paper. It consists of *80%* polyethylene oxide and *20%* polypropylene oxide. It has a mean molecular weight of *8350 g/mol* and a relative solubility of *>10%* in water at *25 °C*. The critical micelle concentration (*CMC*) is reported from Sigma Aldrich with *0.04 mM* in a water solution at *20* to *24 °C* [54].

At a concentration below the *CMC*, surfactant molecules are freely suspended in the bulk fluid or they position themselves at free surfaces. Therefore, the surface tension of the liquid decreases with increasing concentration of surfactants. Above the *CMC*, surfactant molecules start forming spherical aggregates due to their affinity to phase separation. These formed entities are called micelles[55]. For PF68, the *CMC* corresponds to a concentration of *0.33 g/L* in aqueous solution.

For the following mass transfer measurements PF68 concentrations of *0.02 g/L* up to *3 g/L* were examined – as they are similar to concentrations in commercially available media[23-25]. In order to create a solution with the equivalent concentration in the vessels, a *10 wt%* stock solution was used.

3 THEORETICAL ASPECTS

3.1 Pluronic® F68 and Impact on Cell Culture

The entire fluid dynamics in mixed and aerated tanks is influenced by surface-active substances. As mentioned before, animal cell lines are sensitive to shear stress. One of the most important substances for the protection of cells to shear stress is PF68. Therefore, in most of the commercially available media PF68 is an essential component. Mainly, two mechanisms have been outlined in literature to describe the shear protective effects of PF68.

The first mechanism deals with the accumulation of surfactants at the gas-liquid interface. As known from literature, the most damaging factor in mechanically-agitated and sparged bioreactors, mostly used for large scale animal cell culture, is the bubble breakup at the liquid surface.[13, 27] Bubble rupture results in very high accelerations and therefore high energy dissipations, which can damage cells. Additionally cells can be ejected from the media to the reactor headspace or to a foam layer on top of the liquid.[13, 15, 18, 56] PF68 decreases bubble size and the surface tension σ is usually reduced by this compound. In addition, the bubble interface layer is stabilized and the time required for thinning the liquid film around the bubble during the bursting process will be enhanced. If there are cells attached on the bubble surface, they have more time to "slip" again into the media. A more detailed description and discussion can be found by Wu[31]. It was also demonstrated that the attachment of cells to the bubble surface layer would be reduced by addition of PF68.[57, 58]

A second protection mechanism described a direct effect on the stability of the cells.[30, 58, 59] Here, the cell plasma membrane is stabilized by the addition of PF68. Gigout et al. [33] have demonstrated for CHO and cartilage cells with an fluorescence technic that Pluronic® F68 can enter the cell membrane and accumulate in the endocytic pathway.

Recent studies showed that PF68 can have an impact on the production and the glycosylation of proteins.[60] They also showed that the utilization of two major carbon substrates is increased by the addition of PF68.

3.2 Models of Mass transfer

A lot of gas liquid mass transfer models and theories have been published in literature. In order to prepare a structured discussion of the results in this article a short review of the most common models and general principles is outlined in the following paragraph.

In principle, the kinetics of the dissolved oxygen concentration in liquid media can be described by first Fick's law, which describes the molar flux (J) across the area of a thin liquid film:

$$J = \frac{dn_{mol}}{A \cdot dt} = -D_{sw} \frac{dc}{dz} = -k_T \cdot \Delta c \tag{7}$$

Here, (n_{mol}) is the amount of the solute and (A) is the specific area for the diffusion process. Using the diffusion coefficient (D_{sw}), the molecular flux can be described as a concentration difference over distance z. By differentiating this, we get the integral or total mass transfer coefficient (k_T) and the total concentration difference (Δc). To obtain the volumetric mass transfer coefficient ($k_L a$), the mass transfer must be related to overall mass transfer area.

$$a = \frac{A_B}{V_L} \tag{8}$$

Here the specific interfacial area (a) is defined as ratio of bubble surface area (A_B) to the volume of the liquid (V_L).

The most common model to describe the mass transfer through a two phase boundary is the Two-film theory by Lewis and Whitman [38]. In this model, on both sites of the phase boundary a stationary, laminar, fluid film is build up. Within these films, the oxygen is transferred only by molecular diffusion. The total mass transfer coefficient (k_T) is a combination of the mass transfer on the liquid site (k_L) and the gas site (k_G). Both parts are functions of the diffusion coefficient (D_i) and the thickness of the laminar layer (δ_i) at the phase boundary:

$$\frac{1}{k_T} = \frac{R \cdot T}{k_G \cdot He_C} + \frac{1}{k_L} \approx \frac{1}{k_L} \qquad \text{with} \quad k_i = \frac{D_i}{\delta_i} \tag{9}$$

The gas liquid mass transfer is characterized by the mean free path lengths in liquid media and significantly slower compared to diffusion in gas phase (10^4-10^5 slower diffusion coefficients). Therefore, the mass transfer coefficient on the gas side is high, and the mass transfer on the liquid side becomes the limiting factor.

The Two-film model, however, assumes strictly stationary films and describes the transport operation only by means of linear concentration gradients. As an alternative a penetration model was presented by Higbie[39]. This allows, in contrast to the model by Lewis and Whitman, a movement on the phase boundary. According to his theory, it is assumed that on both sides of the phase boundary, fluid elements in a specific volume have for a certain period of time (τ) contact with each other. Each of these fluid elements results in mass transfer during the contact time. An exchange against new fluid elements is assumed after a certain dwell time. Like Higbie[39] the liquid film mass transfer coefficient (k_L) can be calculated by:

$$k_L = \sqrt{\frac{4 \cdot D_{sw}}{\pi \cdot \tau}} = \sqrt{\frac{4 \cdot D_{sw} \cdot v_S}{\pi \cdot d_B}} \tag{10}$$

where (D_{sw}) is the diffusion constant, (v_S) is the bubble velocity relative to the liquid phase and (d_B) is the bubble diameter. The molecular diffusion, therefore, is a non-stationary process and a function of the contact time of the fluid elements. The diffusion constant becomes influenced by media properties, e.g., viscosity, as can be seen by the Stokes-Einstein equation.

$$D_{sw} = \frac{k_B \cdot T}{6 \cdot \pi \cdot \mu \cdot R_0} = \frac{k_B \cdot T}{f} \tag{11}$$

Here, (k_B) is Boltzmann's constant, (T) the temperature, (μ) the viscosity of the liquid and (R_0) is the solute radius (usually half of the mean free length of path). More details of methods to calculate diffusion constants can be found elsewhere.[61]

The penetration theory after Higbie assumes a uniform residence time (τ) for the mass transfer which is not the reality. To remedy this shortcoming, a frequency distribution of the contact times for the individual fluid elements at the phase boundary was introduced by Danckwerts[40] in his surface renewal theory. The transport through the specific phase boundary by molecular diffusion takes place during this different contact times. Turbulent movement of volume elements was assumed as well. An experimental determination of this time distribution functions is almost impossible and so the mathematical modeling of this distribution function is difficult.

The measured effect of Pluronic® F68 on mass transfer from this study will be discussed in reference to these theories.

4 EXPERIMENTAL INVESTIGATIONS AND DISCUSSIONS

4.1 Mass transfer of different aeration systems at different Pluronic® concentrations

The influence of Pluronic® F68 on the mass transfer coefficient k_La was measured as described in 2.2. In Figure 4 an exemplary result of the effects of different PF68 concentration on volumetric mass transfer coefficient k_La is shown. The results were determined in the described *400 L* system for a mechanical power input of *60 W/m³* and an aeration rate of *20 L/min* for the tested aeration systems depicted in Figure 2.

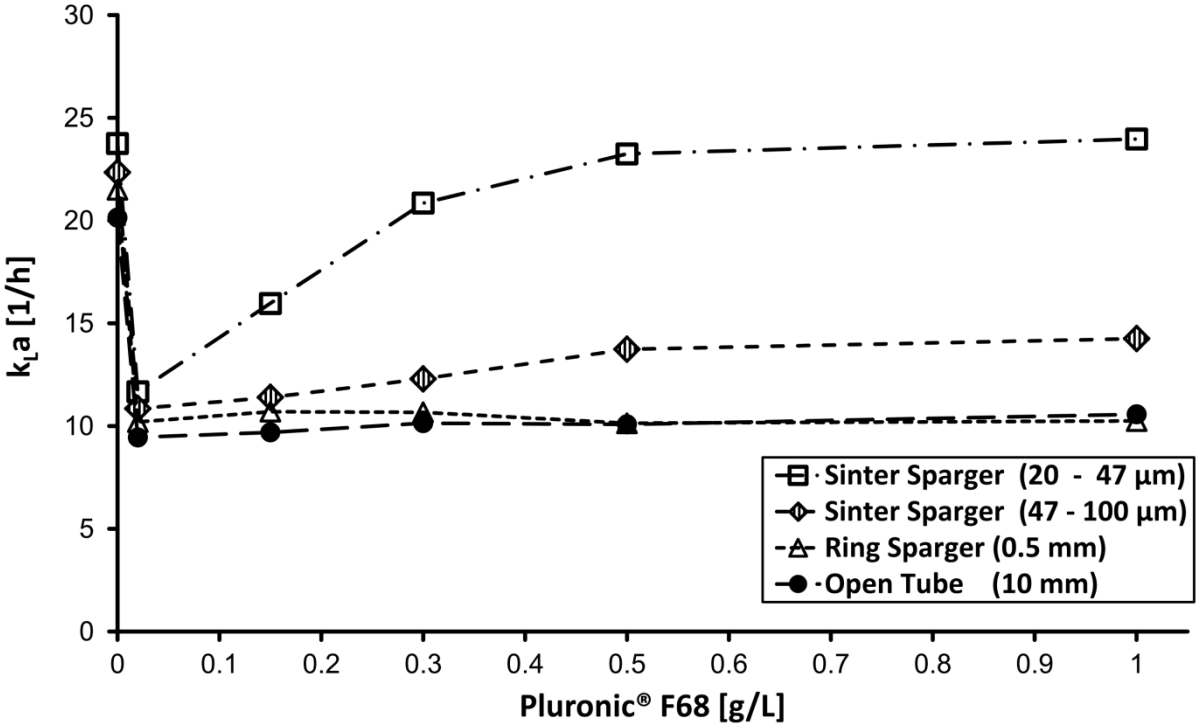

Figure 4. Mass transfer coefficient k_La as a function of the Pluronic® F68 concentrations for different sparger systems. The experiments were conducted at power inputs of *60 W/m³* and an aeration rate of *20 L/min*.

The results clearly indicate a significant impact of PF68 on the mass transfer. A decrease of the volumetric mass transfer coefficient (k_La) in all aeration systems of at least *50%* at the very low PF68 concentrations of *0.02 g/L* compared to the reference without addition of PF68 was observed. This concentration is far below the reported critical micelle concentration (*CMC*) of *0.33 g/L*.

Interestingly, with further increase in concentration of PF68, a significant increase of k_La of the sintered spargers was seen. At *1 g/L* PF68, the (k_La) value for the sinter sparger with the smallest pore size (*20-47 µm*) returned to values similar to those measured under conditions without PF68. This behavior could not be seen for the ring sparger or the open tube. There the k_La reducing effect of PF68 remained for the entire tested concentration range.

Similar trends could be observed when the reactor is operated as a bubble column without agitation even at higher PF68 concentrations of up to *3 g/L* as shown in Figure 5.

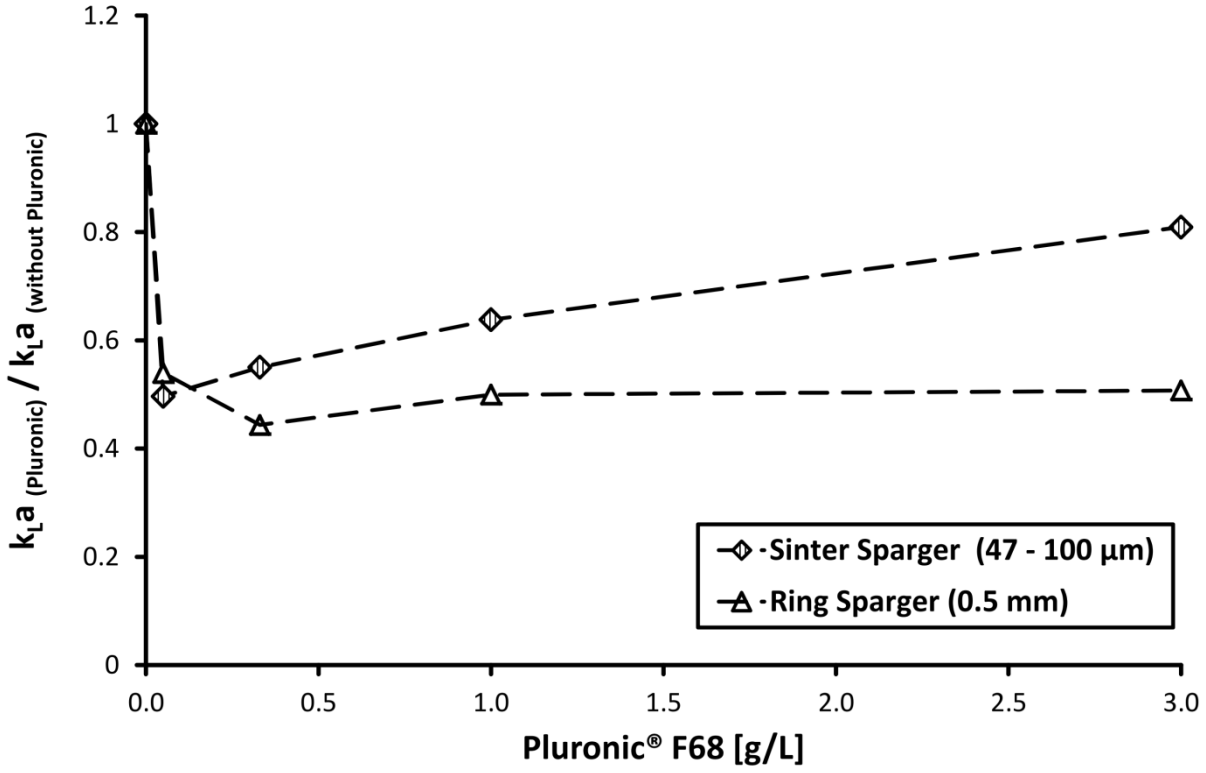

Figure 5. Relative mass transfer coefficients in bubble column mode (without agitation) for ring sparger (*18 holes, ∅0.5 mm*) and sinter sparger (*47-100 µm*) at different Pluronic® F68 concentrations. The shown results were conducted an aeration rate of *1 L/min*.

A clear influence of PF68 concentration was observed during these experiments. The observed drop of the volumetric mass transfer coefficient (k_La) is influenced by the size of the specific interfacial area (a) or by the liquid mass transfer coefficient (k_L). Since no other parameters were changed, a direct relationship between k_La and the PF68 concentration even below the *CMC* can be concluded. In order to allow a differentiation of the different parameters affecting k_La, the influence on the bubble size is assessed in the next section.

4.2 Analysis of Bubble Size and Appearance

It is well known from literature that surfactants reduce the surface tension at gas liquid interface. This effect leads to the formation of smaller bubbles of a more uniform and round shape[44] and therefore they have a direct impact on gas hold up and the specific area available for mass transfer. To describe the shift of mean bubble diameter (d_{B50}) by changing surface tension Liepe[44] has published the following correlation:

$$d_{B50} = 1.3\sqrt{\frac{\sigma}{g \cdot \rho_L}} \tag{12}$$

where (σ) is the surface tension, (g) is the gravity and (ρ_L) is the liquid density.

As shown by Murhammer[24] the surface tension of an aqueous solution will be reduced from *72 mN/m* to *50 mN/m* by addition of *1 g/L* PF68. Typically, the surface tension decreases rapidly up to the critical micelle concentration. At concentrations above *CMC*, only a modest reduction of surface tension

is observed. We can assume that the surface tension of *1 g/L* PF68 solution is nearly the same as that of *0.33 g/L* PF68 solution. The parameters published by Murhammer[24] results, according to equation 12, to a reduction in mean bubble diameter from *3.52 mm* to *2.93 mm*. For a gas volume of *1 L*, this leads to an *20 %* increase of mass transfer area from *1.71 m²* up to *2.04 m²*.

Hence, increased mass transfer area (a) should rather increase the overall mass transfer coefficient (k_La). However, as described in Figure 5 a significant drop was observed below concentrations of *0.1 g/L*. As this is contrary to the theory by Murhammer[24], further measurements of the real bubble diameters under these process conditions were conducted.

Since the approximation in shift of mean bubble diameter (d_{B50}) is a relative measure, further experimental analysis of bubble appearance and diameters were performed for the sinter spargers as described in 2.1. Figure 6 shows the visual observations of the bubble size and shape for the tested sinter spargers.

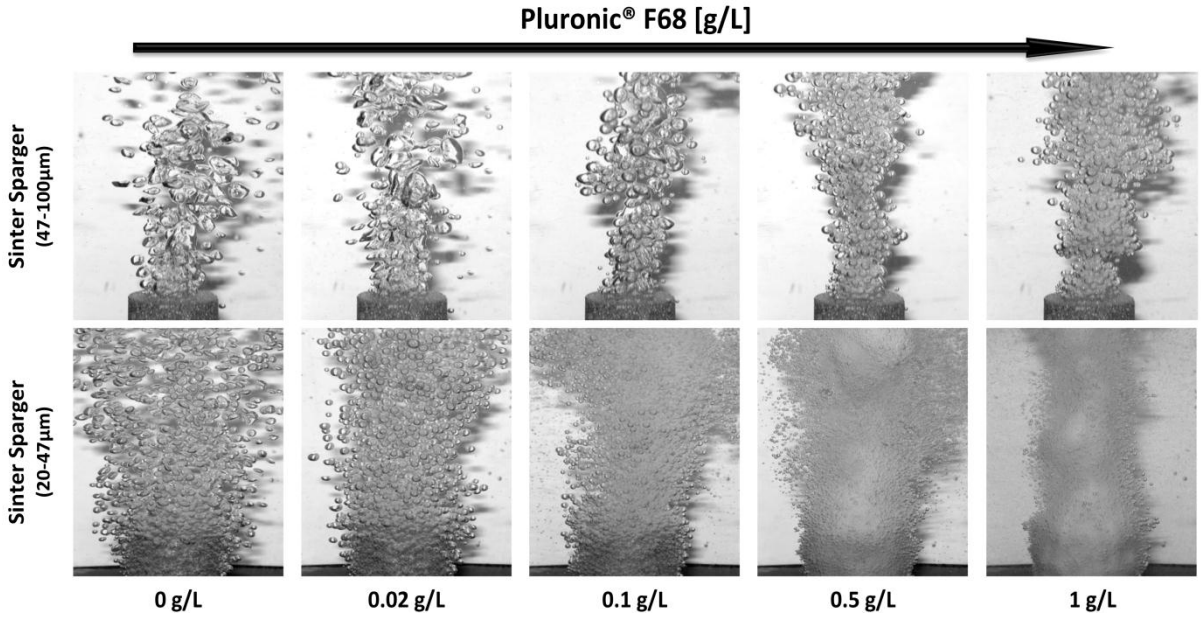

Figure 6. Bubble size and appearance at different Pluronic® F68 concentration at an aeration rate of *1 L/min.*

Without any addition of PF68, small ellipsoid bubbles are visible. At the concentration of *0.02 g/L* no significant change of the bubble diameter or shape is visible. The first impact on bubble sizes and appearance can be seen at *0.1 g/L*. At higher concentrations of *0.5* and *1 g/L*, smaller and rounder bubbles become visible. At *1 g/L* single bubbles are not visible anymore and it can be characterized as a swarm of micro bubbles. Therefore, the non-coalescence characteristic of the media seems to be fully developed. The impact of PF68 above *0.1 g/L* is leading to significantly smaller bubble size and therefore contributing to the significant increase of the mass transfer coefficient shown in Figure 4. Furthermore, no obvious changes in bubble size or shape could be seen for concentrations below *0.1 g/L*. Hence, the observed decrease in k_La can only be attributed to a large decrease in the intrinsic mass transfer coefficient (k_L). This was also confirmed by the determination of the Sauter diameter (d_{P32}).

In order to quantify the effects of PF68 on bubble diameter and hence the volume specific area (a), the Sauter diameter (d_{P32}) was determined by a Laser-light scattering system. The sinter sparger (*47-*

100 µm) and the ring sparger, as described above, were installed and tested in the equipment described in 2.2.2. The sintered sparger with smaller bubble size (*20-47 µm*) could not be analyzed since the bubble swarm was too dense for the measurement equipment. In Figure 7, the determined Sauter diameters at *1 L/min* sparging rate are plotted against the PF68 concentration.

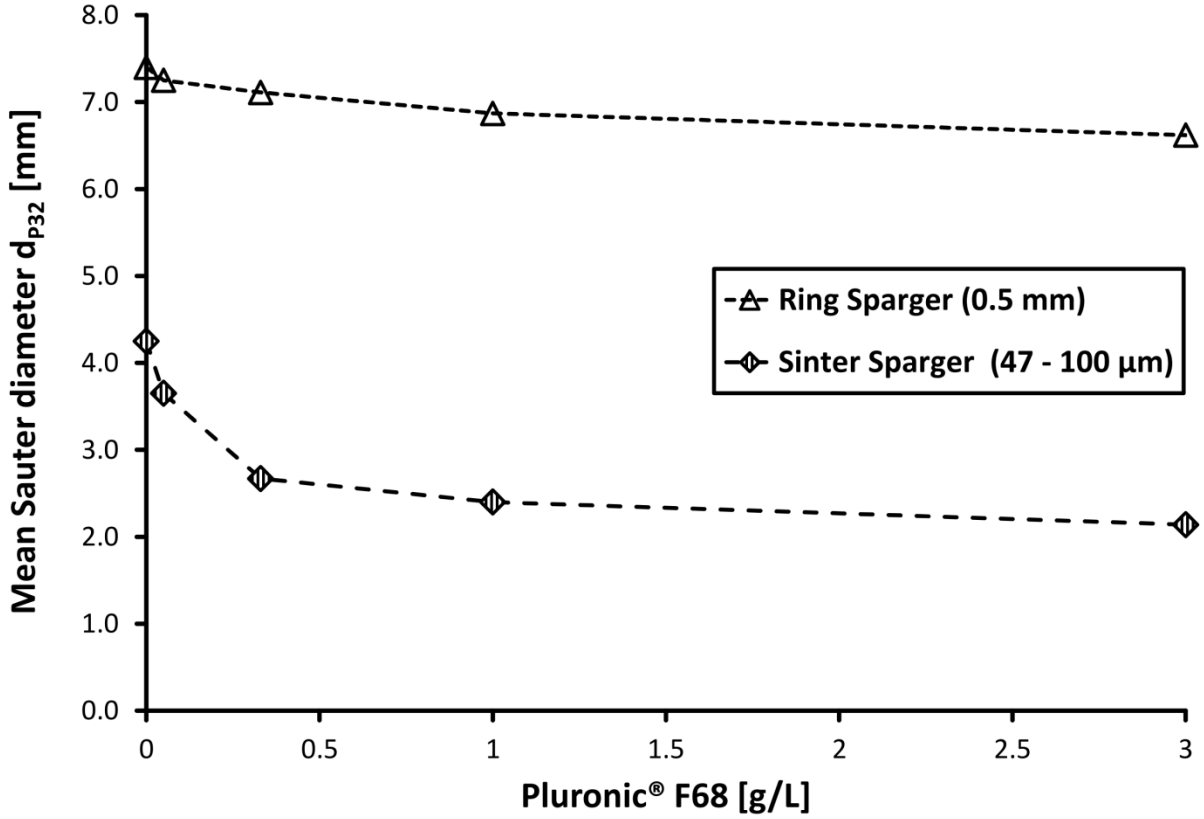

Figure 7. Influence of the Pluronic® F68 concentration on the Sauter diameter of bubbles measured for a ring sparger (*18 holes, ∅0.5 mm*) and a sinter sparger (*47-100 µm* pore size) in a rectangular bubble column. The sparging rate for these measurements was *1 L/min*.

A decrease of the Sauter diameter can be seen in both cases for the entire concentration range. The increasing PF68 concentration in the medium lead to a reduction of the primary bubble size by reducing the surface tension and thus increasing specific surface area (*a*) for mass transfer. The strongest decrease of bubble size was seen in the concentration range of *0* to *0.33 g/L*.

At *0.33 g/L* PF68, the Sauter diameter for bubbles of the ring sparger decreases by *3.9%*, which leads to an increase in specific surface area by *4.1%*. In the case of the ring sparger under the same conditions, bubble diameter is decreased by *37%* leading to an increase of *a* by *59%*. At higher PF68 concentration the bubble diameters and therefore the specific interfacial area (*a*) is relatively constant.

Under the assumption of a constant mass transfer coefficient (k_L), an increase in the overall mass transfer coefficient (k_La) should be expected. However, in the experiments conducted, a *50%* drop of in overall mass transfer (see Figure 4) was measured. Therefore, it can be concluded, that there is a strong negative impact of PF68 addition on the mass transfer coefficient (k_L). The significant decrease of the mass transfer coefficient by *50%* cannot be attributed to the bubble size and subsequently to the specific mass transfer area (*a*).

4.3 Discussion of k_L impact

Following the mass transfer model of Lewis and Whitman [38], the reduced volume specific mass transfer coefficient (k_La) can be explained by either a significant decrease of the specific surface area (a) or an change of mass transfer coefficient (k_L) due to increased surface layer thickness (δ). No significant impact on the bubble size was measured for the addition of *0.02 g/L* PF68 and, furthermore, no impact on other parameters which influence diffusion coefficient (e.g. viscosity, temperature) is expected at this low PF68 concentration (see Eq. 10). Hence, two remaining factors should be analyzed as possible root cause for the decreased mass transfer at low PF68 concentration: The influence of the increased gas hold up affected by the bubble size and an increased surface layer thickness (δ).

By using the measured bubble sizes and following equation 13 the gas hold up is not expected to increased significantly at low PF68 concentration. Moreover, a reduction of *50%* of the overall mass transfer cannot be explained by this phenomenon.

$$\varepsilon_G = a\frac{d_{P32}}{6} \tag{13}$$

Thus, a significant increase in surface layer thickness (δ) remains the only plausible root cause for the observed phenomenon. Looking at the used concentration of *0.02 g/L* PF68, a full formation of an additional layer around the bubble is unreasonable since this concentration is significantly lower than the critical micelle concentration described in literature. Furthermore, surfactant molecules like Pluronic® will be moved with the liquid velocity along the surface of the gas bubble to the bottom, as shown in Figure 8.[44]

Therefore a significant increase in thickness of the boundary layer(δ), according to the two-film theory, is highly unrealistic. Hence, the measured effect of a *50%* reduction of mass transfer at low concentrations cannot be completely explained by an inhibition of molecular diffusion with the model of Lewis and Whitman[38]. Since, PF68 is forming monolayers, a further increase of (δ) would not be expected once a full layer has been developed. This will lead to a constant mass transfer coefficient (k_L).

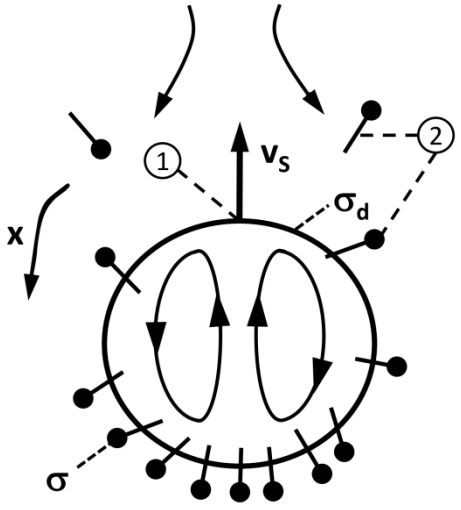

Figure 8. Sketch view of accumulation of surfactant molecules in the bubble phase boundary layer. ① = stagnation point; ② = surfactant molecules with hydrophilic head; σ_D = dynamic surface tension; σ = static surface tension; v_s = bubble velocity (according to Liepe[44])

As not described in the sketch of Liepe [44], the circulation of the bubble surface layer will be inhibited and disturbed through the accumulation of PF68 molecules. This change from a circulating to a more rigid bubble interface was described by Jordan et al. [62]. This information allows the hypothesis that the measured effects can be better explained by the models of Higbie[39] or Danckwerts[40]. Due to the reduced circulation of the phase boundary layer, it comes to increased contact times of the fluid elements and, therefore, the liquid film mass transfer coefficient (k_L) will be reduced significantly.

Furthermore, Jordan et al.[62] also describes a decrease of bubble velocity for bubbles with a diameter of *1.5 mm* from *0.35 m/s* for pure water down to *0.17 m/s* for water with *0.025 g/L* PF68, due to the accumulation of surfactants on the bubble surface layer. The increased bubble residence time should result in increased mass transfer. However, the opposite is the case, as seen in Figure 4 and Figure 5. Therefore, we can conclude, that at low surfactant concentrations the change of mass transfer coefficient (k_L) is dominating the mass transfer which can be explained best by the models of Higbie[39] or Danckwerts[40].

Using the measured mass transfer coefficients (k_La) from the bubble column experiments and the results from the bubble size measurements allows the calculation of (a) and therefore an approximation of (k_L). The calculated results are shown in Figure 9 and Figure 10.

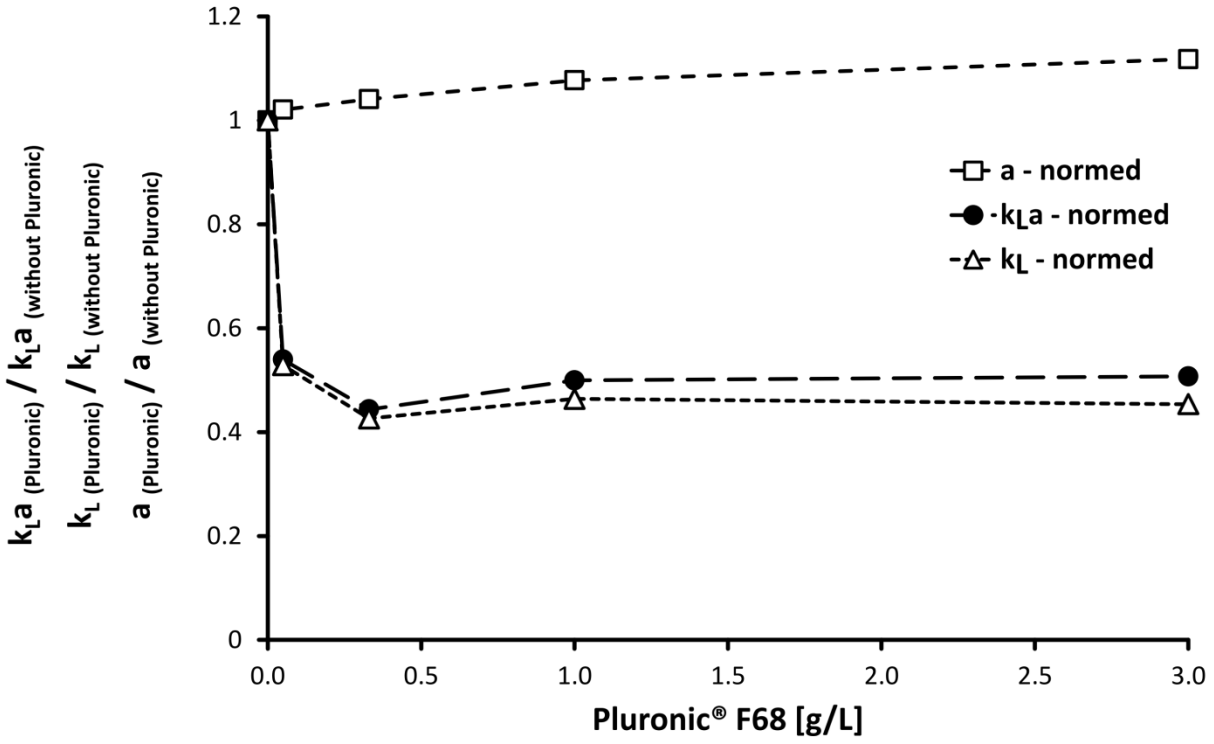

Figure 9. Influence of Pluronic® F68 concentrations on the volumetric mass transfer coefficient (k_La), the liquid side diffusional resistance (k_L) and the specific interfacial area (a) for a ring sparger with 0.5 mm hole diameter. All values were normalized to the values without Pluronic® F68 addition.

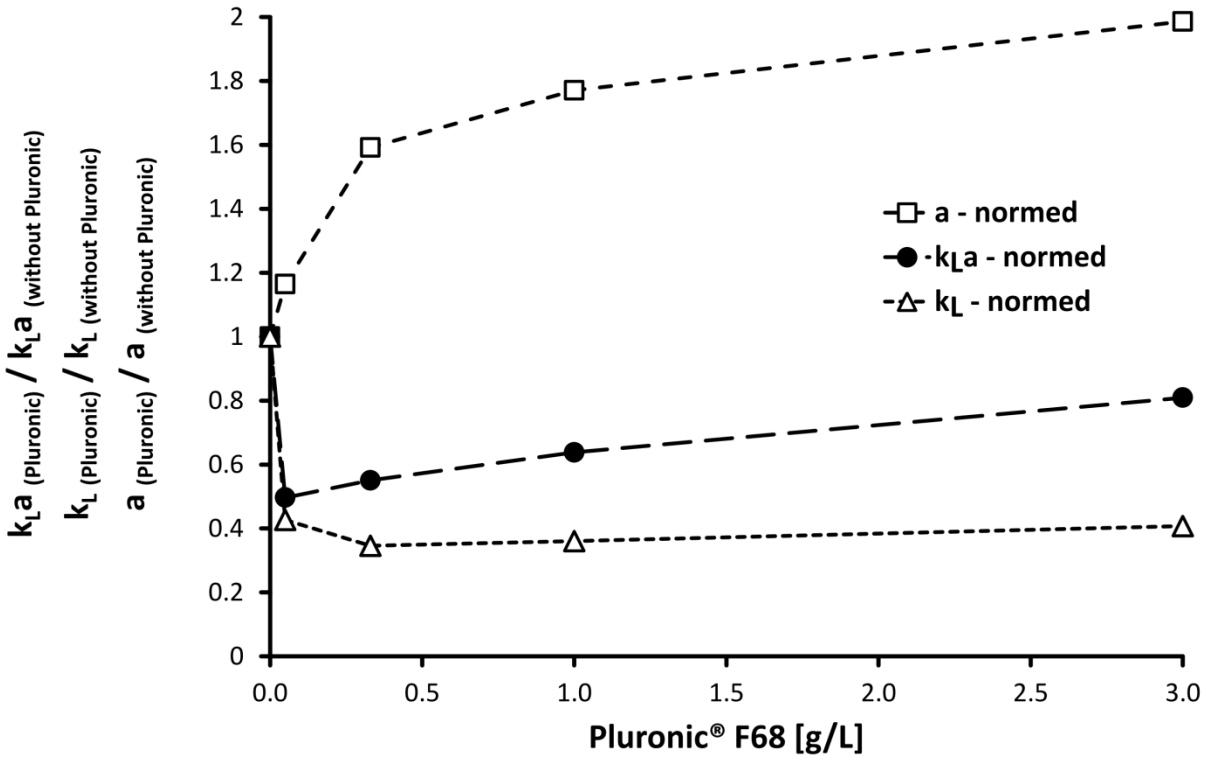

Figure 10. Influence of Pluronic® F68 concentrations on the volumetric mass transfer coefficient (k_La), the liquid side diffusional resistance (k_L) and the specific interfacial area (a) for the sinter sparger (*47-100 µm*). All values were normalized to the values without Pluronic® F68 addition.

For the ring sparger (Figure 9) as well as the sintered sparger (Figure 10) a strong reduction of the mass transfer coefficient (k_L) by up to *60-70%* is visible at low concentrations well below the critical micelle concentration. With increasing PF68 concentration between *0.02* to *0.3 g/L*, the effect of reduced mass transfer coefficient values (k_L) is more dominated and stabilized by the formation of closed single layer and, therefore, by the thickness of the interfacial area. Above the concentration of *0.3 g/L* PF68, which is close to the *CMC* (*0.33 g/L*) reported by the vendor Sigma Aldrich, no significant impact on mass transfer coefficient (k_L) can be seen anymore. One reason might be the tendency of Pluronic® to form so called Langmuir monolayers at the gas liquid interface. Due to the critical micelle concentration of surfactants the formation of multi layers is energetically unfavorable[63].

For higher PF68 concentrations (*>0.3 g/L*), the influence of contact times on the k_L value, used in models by Higbie[39] or Danckwerts[40], is considerably reduced due to the reduced surface movement. The mass transfer model by Lewis and Whitman[38] could also be used for solutions with PF68 concentrations above the *CMC* since the formation of the full PF68 layer is reached. Due to the rigid bubble surface, the diffusion process through the layer with thickness (δ) becomes the rate-determining step.

As shown before, the impact of bubble size and increase in surface area is now dominating the mass transfer coefficient. As can be seen in Figure 9 and Figure 10, especially for the *20-47µm* sinter sparger, an increase in the volume specific area (a) translates directly into an increase in the volumetric mass transfer coefficient (k_La). Especially the sinter spargers show a decrease in bubble diameters and increase in volume specific area due to minimized coalescence and lower surface tension caused by the surfactant. As result, the volumetric mass transfer coefficient (k_La) increases significantly with

increasing PF68 concentrations but it does not result in higher volumetric mass transfer values (k_La) compared to conditions without PF68.

5 CONCLUSIONS

A clear dependence of bubble sizes and mass transfer on the concentration of Pluronic® F68 above and below the critical micelle concentration was observed in this study. At lower concentrations of *0.02 g/L*, a decrease in the overall mass transfer coefficient (k_La) of *50%* was detected, which could be attributed to an impact on mass transfer coefficient (k_L). Following the theories of Lewis and Whitman [38] a plausible explanation by increasing the thickness of the boundary layer does not seem feasible. However, with theories of Higbie[39] or Danckwerts[40], the disturbance of fluid element movement at the bubble surface can explain the decrease in mass transfer. Furthermore, an impact up to *0.3 g/L* in bubble columns could be found and explained by bubble size measurements. Above these concentrations most likely a full monolayer around the bubble allows the application of the Lewis and Whitman[38] model. At concentrations above *0.1 g/L* however, a significant change in bubble appearance and bubble sizes is dominating the overall mass transfer by increasing gas holdup and, more importantly, increasing the surface area available for mass transfer. Visual analysis and calculated Sauter diameters show a significant decrease up to *40%* in terms of sinter sparger (*47-100 µm* pore size) corresponding to an *80%* increase in specific mass transfer area (a).

The approximation of the mass transfer coefficient (k_L) and the interfacial area (a) in the column experiment clearly show the dominating effect of mass transfer coefficient (k_L) for the entire concentration range tested. With increasing amounts of PF68, the specific interfacial area (a) increases. The magnitude of increase of (a) is dependent on the sparger type and appears to be the dominating factor for the increase in k_La observed at higher PF68 concentrations.

One could conclude that concentrations between *1 g/L* and *3 g/L* are more favorable. A critical or optimal concentration for cell cultures cannot be concluded from these experiments since this might be process and media-dependent. However, the fundamental mechanics of oxygen mass transfer under the influence of Pluronic® F68 were described in this study. The mass transfer of carbon dioxide is a further important factor for animal cell culture processes. As next steps, the influence of PF68 on CO_2 mass transfer will be evaluated since the saturation and mass transfer coefficients are mostly dominated by bubble size and bubble residence time[10] and therefore the effect for stripping CO_2 might be more relevant in this context.

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Conflict of interest statement

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6 NOMENCLATURE

Symbol	Description	Unit
a	volume specific mass transfer area	[m²/m³]
A	interfacial of stagnant boundary layer	[m²]
A_B	bubble surface area	[m²]
c^*	saturation concentration in the liquid	[mol/L] or [%]
c_L	current concentration at the liquid	[mol/L] or [%]
$c_{L,0}$	concentration at the liquid at t=0 s	[mol/L] or [%]
D	stirrer diameter	[m]
d_B	bubble diameter	[m]
d_{B50}	mean bubble diameter	[m]
D_i	diffusion constant	[m²/s]
δ_i	thickness of the laminar layer	[m]
d_{P32}	Sauter diameter	[m]
D_{sw}	diffusion constant gas in water	[m²/s]
ε_G	gas holdup	[-]
f	friction function	
g	gravity	[m/s²]
He_C	Henry's constant	[bar*L/mol]
H	Filling Height	[m]
J	molecular flux across an area	[mol/(m²*s)]
k_B	Boltzmann constant	[J/K]
k_G	total mass transfer coefficient	[m/s]
k_i	mass transfer coefficient	[m/s]
k_L	mass transfer coefficient at the liquid	[m/s]
k_La	volumetric mass transfer coefficient	[1/h]
k_T	total mass transfer coefficient	[m/s]
$M_{effective}$	effective torque	[Nm]
M_{empty}	torque at empty vessel	[Nm]
M_{load}	torque at loaded vessel	[Nm]
μ	viscosity of the liquid	[Pa*s]
n	stirrer speed	[1/s] or[rpm]
n_{mol}	amount of solute	[mol]
OTR	oxygen transfer rate	[mol(O2)/L*h]
P	Power	[W]
R	gas constant	[J*mol^{-1}*K^{-1}]
R_0	mean free path length	[m]
ρ_L	liquid density	[kg/m³]
σ	surface tension	[N/m] or [kg/s²]

σ_d	dynamic surface tension	[N/m] or [kg/s²]
T	temperature	[K]
t	time	[s]
T_D	tank diameter	[m]
τ	time constant	[s]
t_{P63}	probe response time	[s]
V	Volume	[m³] or [L]
v_S	bubble velocity	[m/s]
w	baffle width	[m]
x	liquid velocity	[m/s]
Δc	concentration difference	[mol/L] or [%]

Abbreviations:

ccd	charged coupled device
CHO	Chinese hamster ovary cells
CMC	critical micell concentration
CO_2	carbon dioxide
MFC	mass flow controller
NaCl	sodium chloride
O_2	oxygen
PF68	Pluronic® F68
Po	power number
rpm	round per minute
TQS	torque sensor
vvm	volume per volume and minute
sLpm	standard liter per minute

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CHAPTER 5

A TEST FACILITY FOR FRITTED SPARGERS OF PRODUCTION-SCALE-BIOREACTORS

ABSTRACT: The production of therapeutic proteins requires qualification of equipment components and appropriate validation procedures for all operations. Since protein productions are typically performed in bioreactors using aerobic cultivation processes air sparging is an essential factor. As recorded in literature, besides ring spargers and open pipe, sinter frits are often used as sparging elements in large scale bioreactors. Due to the manufacturing process these frits have a high lot-to-lot product variability. Experience shows this is a practical problem for use in production processes of therapeutic proteins, hence frits must be tested before they can be employed. The circumstance of checking quality and performance of frits as sparging elements was investigated and various possibilities have been compared. Criteria have been developed in order to evaluate the sparging performance under conditions comparable to those in production bioreactors. The oxygen mass transfer coefficient (k_La) was chosen as the evaluation criterion. It is well known as an essential performance measure for fermenters in the monoclonal antibody production. Therefore a test rig was constructed able to automatically test frit-spargers with respect to their k_La-values at various gas throughputs. Performance differences in the percent range could be detected.

KEYWORDS: fritted spargers, bubble aeration, oxygen supply, animal cell bioreactors

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CURRICULUM VITAE

Persönliche Daten

Name:	Christian Philipp Sieblist
Geburtsdatum:	07.07.1973
Geburtsort:	Nordhausen, Germany
Nationalität:	deutsch

Bildung

2005 - 2008	Promotionsstudent am Zentrum für Bioverfahrenstechnik der Martin-Luther Universität Halle-Wittenberg, (05/2005 – 04/2007 Promotionsstipendium des Landes Sachsen-Anhalt, Martin-Luther-Universität Halle-Wittenberg)	Halle (Saale)
2005 - 2006	Teilnahme am Projekt REGIO●START – Führungskräfte für die regionale Wirtschaft, des Landes Sachsen-Anhalts inkl. Ausbildung zum zertifizierten Projektmanager, Abschluss: Projektmanager Level D gemäß GPM Richtlinie, Zert.-Nr.: IT-P 03-27, Otto-von-Guericke-Universität Magdeburg	Magdeburg
1999 - 2005	Studium im Studiengang Werkstoffwissenschaften, spez. Werkstofftechnik, Abschluß als Diplomingenieur für Werkstoffwissenschaften, Martin-Luther-Universität Halle-Wittenberg	Halle (Saale)
1998 - 1999	Studium im Studiengang Rechtswissenschaften, Martin-Luther-Universität Halle-Wittenberg	Halle (Saale)
1994 - 1998	Allgemeine Hochschulreife, Abendgymnasium und Kolleg	Halle (Saale)
1990 - 1994	Ausbildung zum Feinmechaniker für Feinwerk- und Gerätetechnik, Abschluß mit Gesellenbrief, Max-Planck-Institut für Mikrostrukturphysik und Elektronenmikroskopie	Halle (Saale)
1989 - 1990	Polytechnische Oberschule für Körperbehinderte Nikolai Ostrowski	Halle (Saale)
1980 - 1989	Polytechnische Oberschule N. K. Krupskaja	Halle (Saale)

Tätigkeiten

seit 2009	Roche Diagnostics GmbH, Pharmaceutical Biotech Production, Penzberg	Penzberg
2007 - 2008	Zentrum für Bioverfahrenstechnik, Institut für Biochemie und Biotechnologie, Martin-Luther-Universität Halle-Wittenberg	Halle (Saale)
1998 - 1999	Ingenieurbüro für Prüf- und Baustatik U. Klein	Halle (Saale)
1994	Max-Planck-Institut für Mikrostrukturphysik und Elektronenmikroskopie	Halle (Saale)

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Halle (Saale), den 29. Oktober 2012

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Christian Sieblist