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Gas mass transfer in syngas fermentation broths is enhanced by ethanol

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ABSTRACT

In gas fermentations (using O₂, CO, H₂, CH₄ or CO₂), gas-to-liquid mass transfer is often regarded as one of the limiting processes. However, it is widely known that components in fermentation broths (e.g., salts, biomass, proteins, antifoam, and organic products such as alcohols and acids) have tremendous impact on the volumetric mass transfer coefficient k_La . We studied the influence of ethanol on mass transfer in three fermentation broths derived from syngas fermentation. In demineralized water, we observed that the addition of ethanol, the expected product, increased k_La two-fold in the 0–5 g L⁻¹ range, after which near-constant k_La values were obtained. In the fermentation broths, k_La was increased significantly (2–4 fold compared to water) by ethanol supplementation, and to be highly influenced by broth salinity. Our results indicate that k_La is a dynamic parameter in gas fermentation experiments and can be significantly increased due to broth components.

1. Introduction

Many fermentations with gaseous substrates (e.g., O₂, CO, H₂, CO₂, CH₄) are considered as promising conversion processes for a multitude of useful products (e.g., succinic acid, ethanol, butanol, hexanoic acid, lactic acid) [1–3], of which several are established in industrial practice [4,5]. For many of these processes, poor gas-to-liquid mass transfer and low dissolved gas concentrations have been identified as a limiting factor [6–9]. Based upon that, a lot of research, for example in syngas fermentation, is focused on increasing the volumetric mass transfer coefficient (k_La) by developing innovative reactor configurations [6,10,11]. Understanding of the k_La values obtained is essential in the gas fermentation field.

For a long time, it has been known that medium components and products (such as salts, acids, alcohols, surfactants, biomass and antifoam) can significantly affect k_La [12–16]. For a broad range of alcohols in water, a 5-fold increase in k_La was observed in a narrow concentration range [14], which was explained by a decrease in Sauter mean bubble diameter (d_{32}) (from 4 to 1 mm) and a two-fold increase in the gas hold-up (ϵ_G) [17]. The presence of alcohol decreases the surface tension and stabilizes low-diameter gas bubbles, leading to repulsions and thereby inhibiting coalescence [17,18]. Similar effects on mass transfer were not only observed for alcohols, but also for organic acids, ketones and compounds like lactic acid [12,17,19]. Changes in the gas solubility

at alcohol concentrations relevant for gas fermentations were, however, expected to be negligible [20].

Similar effects have been noticed for electrolytes. Lessard and Zieminski [13] observed significant coalescence inhibition (more than 90%) for different salt solutions with ionic strength above 0.3 mol L⁻¹, resulting in increases in k_La values [14]. The presence of biomass, however, may decrease k_La by absorption to the bubble surface (the so-called “blocking effect”), as well as by increasing the viscosity, which stimulates coalescence [16] and reduces the diffusion coefficient [21]. Dissolved proteins are known to improve mass transfer as they stabilize bubbles and prevent coalescence [15]. Furthermore, in air-water systems, the mass transfer coefficient (k_L) is known to be, amongst others, a function of the bubble diameter: Small bubbles (d_b around 1 mm) have a rigid surface with a k_L around 1·10⁻⁴ m s⁻¹, while larger bubbles ($d_b > 2$ mm) have a mobile surface and k_L between 3 and 5·10⁻⁴ m s⁻¹ [22].

Typically, biomass, salts, proteins, products, and substrates are jointly present in fermentation broth, and might change the medium physical properties (i.e., surface tension, viscosity, density) with respect to pure water. Some empirical relations (Table S1) predict the influence of these properties on k_La [9,23–27]. As these relations have been developed using air-water mixtures without taking into account biomass and salts concentrations, their validity for fermentation broths remains unclear.

The aforementioned studies on mass transfer mostly focus on mixtures with water and one compound of interest, while the joint influence

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Nomenclature		Greek	
<i>Latin</i>		ε_G	Gas hold-up ($\text{m}_G^3 \text{m}_D^{-3}$)
a	Specific surface area (m^{-1})	η	Dynamic viscosity ($\text{kg m}^{-1} \text{s}^{-1}$)
A	Surface area (m^2)	ρ	Density (kg m^{-3})
c	Concentration (mol L^{-1} or g L^{-1})	σ	Standard deviation
d_{32}	Sauter mean bubble diameter (m)	σ	Surface tension (N m^{-1})
d_b	Bubble diameter (m)	Sub- and superscripts	
d_{eq}	Equivalent bubble diameter (m)	a	Semi-major axis
e	Eccentricity (-)	Ac	Acetate
I	Ionic strength (mol L^{-1})	c	Semi-minor axis
k_L	Liquid-side mass transfer coefficient (m s^{-1})	b	Bubble
k_{La}	Volumetric mass transfer coefficient (h^{-1})	D	Dispersion
P	Power (W)	<i>EtOH</i>	Ethanol
r	Radius (m)	G	Gas
$u_{G,s}$	Superficial gas velocity (m s^{-1})	L	Liquid
V	Volume (m^3)	<i>prot</i>	Protein
z	Ionic charge (-)	x	Biomass

of different compounds has hardly been studied. Studies with fermentation broths have only been performed to characterize the effect of the biomass concentration on k_{La} [16] and on d_{32} [28]. In wastewater technology, however, it is common to measure aeration performance in process water (with contaminants and biomass sludge), which is often characterized with the alpha-factor, which relates the k_{La} in process water with clean water [29]. Analyses on the joint influence of broth components in gas fermentations on mass transfer are lacking in the scientific literature, making it challenging to estimate. Knowledge on the most influential parameters and their respective ranges would be essential for accurate prediction in real fermentation broths, both during experiments and modeling. With the growing interest in fermentation and bioprocess design, understanding on the most influential parameters and their respective ranges would be essential for accurate k_{La} prediction.

In this study, we aim to determine mass transfer characteristics (k_{La} , d_{32} , ε_G , k_L) in different fermentation broths to show that there are complex interactions between the compounds present and that this has significant consequences in gas fermentation processes. Syngas fermentation is used as an example of a gas fermentation process, as it is a frequently studied process wherein mass transfer is often mentioned as a factor for poor performance [6,11]. For safety and analytical reasons, oxygen mass transfer is studied, but the same trends are expected for other gases that also have a very low solubility in water, such as CO , H_2 , and CH_4 . The very low concentration of dissolved O_2 is expected not to change k_{La} , d_{32} , ε_G , or k_L . The investigated product is ethanol, since it is a major product in the commercialized syngas fermentation process [30]. First, we will determine the range wherein ethanol addition affects k_{La} . After that, we will study the influence of ethanol on the mass transfer characteristics in five mixtures (water, fermentation medium, and three syngas fermentation broths). The experimentally obtained k_{La} values will be compared with k_{La} values from published empirical relations, after determining the physical properties of the mixtures.

2. Materials and methods

2.1. Influence of ethanol concentration on k_{La}

k_{La} was determined in water-ethanol solutions using the dynamic absorption method [9] in a 1.5 L temperature-controlled stirred tank reactor (STR) with 1 L working volume (Applikon Biotechnology, the Netherlands). After desaturation with pure nitrogen, dissolved oxygen (DO) was measured every second with an AppliSens Dissolved Oxygen probe (Applikon Biotechnology, the Netherlands) while supplying 1 vvm

of air at 800 rpm stirring rate. Experiments were performed at 20 °C and 37 °C and at least in trifold for all demineralized water-ethanol mixtures (0, 2.5, 5, 10, 15, 25 and 50 g L^{-1}).

2.2. Determination of mass transfer characteristics of different mixtures

Several mixtures were tested in a bubble column reactor (BCR): demineralized water, mineral fermentation medium (“medium”), and three fermentation broths derived from syngas fermentation experiments (e.g., “broth-1”). A BCR was used since it enables more detailed analyses on d_b and ε_G than a STR does. The influence of ethanol on the mass transfer characteristics was determined by supplementing with the industrially obtained ethanol concentration (50 g L^{-1}) [31], after experiments without supplemented ethanol. [Supplementary material](#) provides the composition of the mineral fermentation medium as well as the media and methods for cultivation of the fermentation broths (Table S2).

Mass transfer characteristics of the different mixtures were determined in a lab-scale glass bubble column (7 cm internal diameter, 70 cm liquid height) with a multi-orifice sparger (0.6 mm orifice diameter). Experiments were performed with air at a low superficial gas flow velocity of 1.8 m s^{-1} to ensure that flow was homogenous and that the individual bubbles could be pictured for bubble size determination. The liquid temperature was kept at 37 °C. Gas hold-up was determined by measuring the ratio between aerated and unaerated volumes [32] using a ruler at the column wall. k_{La} was determined using the same method as described above, with the oxygen probe located 42 cm above the sparger, at least in triplicate for each mixture.

The bubble size was analyzed using two methods, one for the small bubbles in mixtures with supplemented ethanol and another for larger bubbles in the mixtures without ethanol. During aeration, pictures of the small bubbles were made with a photo-optical endoscopic probe (SOPAT-VF GX 2750) (SOPAT, Germany) with the focal plane at 0.5 mm, located 47 cm above the sparger. From 600 images, between 100 and 1400 bubbles (depending on the mixture) were captured with the Hough circle detection method in the Python OpenCV package, and their diameters were calculated using a camera-specific pixel-to-mm conversion factor. As these bubbles were spherical, the Sauter mean bubble diameter d_{32} was calculated using Eq. 1, in which $V_{b,i}$ is the volume of bubble i , $A_{b,i}$ its surface area and $d_{b,i}$ its diameter.

$$d_{32} = 6 \frac{\sum_i V_{b,i}}{\sum_i A_{b,i}} = 6 \frac{\frac{4}{3}\pi \sum_i (\frac{1}{2}d_{b,i})^3}{4\pi \sum_i (\frac{1}{2}d_{b,i})^2} \quad (1)$$

To obtain the (equivalent) diameter of the larger bubbles, pictures were made with a CANON EOS 200D camera. A metal ruler was placed inside the column to decrease the influence of light refraction and to obtain a pixel-to-mm ratio. With image analysis software (ImageJ) d_b was measured for spherical bubbles, and for spheroidal bubbles the radii of the semi-major r_a and semi-minor axes r_c was measured to determine their eccentricity e and equivalent diameter d_{eq} (Eq. 2). Subsequently, d_{32} was calculated with the obtained (equivalent) diameters of the spherical and spheroidal bubbles using Eq. 1. The two bubble size determination methods were cross-validated using broth-4, see Fig. S1 and Table S3.

$$d_{eq} = 6 \frac{V_{b,i}}{A_{b,i}} = 6 \frac{\frac{4}{3} \pi r_a^2 r_c}{2\pi r_a^2 + \pi \frac{r_c^2}{e} \ln\left(\frac{1+e}{1-e}\right)} \quad \text{with} \quad (2)$$

$$e = \sqrt{1 - \frac{r_c^2}{r_a^2}}$$

After determining d_{32} , $k_L a$ and ε_G , Eq. 3 was used to calculate k_L .

$$k_L = \frac{k_L a}{a} \quad \text{with} \quad a = \frac{6\varepsilon_G}{d_{32}} \quad (3)$$

The standard deviation σ of k_L was evaluated using classical error propagation (Eq. 4). The unpaired t-test with Welch's correction was used to determine statistical significance for all mass transfer characteristics.

$$\sigma_{k_L} = k_L \sqrt{\frac{\sigma_{k_L a}^2}{k_L a} + \frac{\sigma_a^2}{a}} \quad \text{with} \quad \sigma_a = a \sqrt{\frac{\sigma_{\varepsilon_G}^2}{\varepsilon_G} + \frac{\sigma_{d_b}^2}{d_{32}}} \quad (4)$$

2.3. Determination of mixture properties

After aeration in the BCR, the mixtures' physical properties were determined at 37 °C. Density was measured with a benchtop density meter (DMA 5000, Anton Paar, Austria). The dynamic viscosity was determined with a Haake Viscotester 500 (Thermo Fisher Scientific, US) with NV sensor system. Dynamic surface tension was measured using a BPT Mobile tensiometer (KRÜSS Scientific, Germany), at least in duplicate.

The biomass concentration for fermentation broth-2, broth-3 and broth-4 was measured by determination of volatile suspended solids (VSS) concentration in the broth, from 150 mL broth samples [33]. For broth-1, the biomass concentration was obtained by measuring its optical density at 660 nm (OD_{660}). This was converted to VSS concentration using calibration curves previously obtained during cultivation. Acetate and ethanol concentrations in filtered broth samples (0.22 μm pore size, Millipore, Millex-GV, Ireland) were determined using ultra high performance liquid chromatography (UPLC) with an Aminex HPX-87 H column (BioRad, United States) at 50 °C coupled to a refractive index (RI) detector RefractoMax 520 (Thermo Fisher Scientific, US), using 1.5 mmol L⁻¹ aqueous phosphoric acid was used as eluent. Lastly, protein concentrations were determined with the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, US) according to the manufacturer recommendations. From the cultivation media composition (Table S2), ionic strength I was calculated (Eq. 5), from each ion concentration c and charge z .

$$I = \frac{1}{2} \sum_i c_i z_i^2 \quad (5)$$

3. Results and discussion

In this section, we will first present the influence of the concentration of ethanol in water on the $k_L a$, which was determined in a stirred tank reactor (Section 3.1). After that, detailed results for the different

mixtures in a bubble column reactor will be presented (Section 3.2), which will be followed by a discussion on k_L for fermentation broths (Section 3.3). Lastly, a comparison with empirical correlations (Section 3.4) will be performed with the determined physical properties.

3.1. Influence of ethanol on $k_L a$

The influence of the ethanol concentration in water on $k_L a$ has been determined in a stirred tank (Fig. 1). Sharp increases in $k_L a$ values were observed in the lower concentration range, until a plateau was reached (between 5 and 10 g L⁻¹, independent from the temperature). For both temperatures, the maximum $k_L a$ value was about twice as large as it would be without ethanol. Visually, we observed a significant reduction in bubble size upon the addition of ethanol, explaining the increased $k_L a$ value (Fig. S2).

The $k_L a$ value increases roughly 40% between 20 and 37 °C, which corresponds with predictions for this temperature-increase [22,34]. The results obtained for these two temperatures indicate that the temperature-increase and the ethanol addition most likely show an independent influence on $k_L a$. This temperature increase predominantly causes an increase in k_L (by increasing the diffusion coefficient, and reducing the kinematic viscosity [21,35]).

In STRs with added ethanol, similar increases in $k_L a$ have been observed before [36], but not with such a clear plateau formation. The plateau formation in $k_L a$ has been observed for other organic compounds in STRs [14], and has been explained by the constant bubble diameter beyond a certain concentration range [17]. Similar results on plateau formation have been seen in a plunging jet contactor with propanol [37] and in an external-loop airlift reactor with methanol, ethanol and propanol [38].

The ethanol concentration range in which the steep change in $k_L a$ values was observed (0–5 g L⁻¹) corresponds to the range of ethanol titers achieved in lab-scale syngas fermentation experiments, for example [6,39,40]. The rapid ethanol concentration change within this range indicates that $k_L a$ is not a constant but a dynamic parameter during gas fermentation processes. For example, during batch operation, different values of $k_L a$ may apply. Moreover, we recommend to obtain $k_L a$ values using representative fermentation conditions rather than using water, to compare reactor configurations [10,41] or to determine dissolved gas concentrations [42].

Consequently, we expect that $k_L a$ differs between fermentation broths derived from syngas fermentation experiments and synthetic aqueous solutions containing some broth solutes. As more detailed analyses on d_b and ε_G can be performed in BCRs, we will use BCRs in the upcoming sections. Due to the plateau formation by ethanol addition, we will perform experiments with the expected industrial ethanol

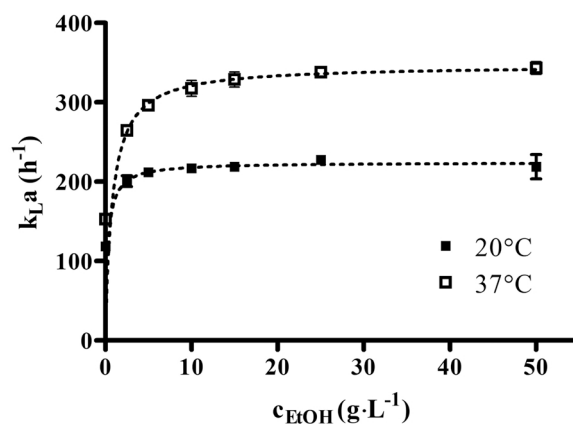


Fig. 1. Influence of the ethanol concentration on $k_L a$, data obtained at 20 °C (filled squares) and 37 °C (open squares) in the stirred tank. Error bars: standard deviations from triplicates.

concentration of 50 g L⁻¹ [31].

3.2. $k_L a$ determination in different mixtures with ethanol

The determined physical properties of the mixtures are shown in Table 1. As the fermentation broths contain only little amounts of ethanol, we supplemented the mixtures with ethanol to reach the industrially relevant concentration (50 g L⁻¹). A clear decrease in surface tension occurs upon supplementation of ethanol. Broth-4 was not included in the table as there was possible interference with the reducing agent during the $k_L a$ -determination (see Section 3.4), such that the $k_L a$ and k_L values could not be predicted reliably.

For all other mixtures, a significant increase in $k_L a$ is observed (Fig. 2a) upon the addition of ethanol ($p = 0.038$). In water, a six-fold higher $k_L a$ is encountered after adding ethanol. Such increases have been obtained before with ethanol [14,43]. This is explained by the decrease in d_{32} (from 2.7 mm to 0.7 mm) and the doubling of the gas hold-up due to the addition of ethanol.

The mineral medium and broth-1 show an increased $k_L a$ compared to the demineralized water. This might be due to their high ionic strength (0.3 mol L⁻¹, Table 1) since little coalescence was expected when ionic strength is above 0.2 mol L⁻¹ [13]. This suggests that $k_L a$ might easily be increased in fermentation broths by increasing ionic strength by slightly changing the mineral medium composition.

Although ethanol decreases the bubble size and makes the bubbles more spherical and rigid (Fig. S3), the beneficial effect of ethanol on $k_L a$ is less pronounced in the mineral medium than in pure water. This lower increase in $k_L a$ can be attributed to a decrease in k_L , which might be due to unresolved complex interactions between salts and ethanol in the boundary layers.

For all fermentation broths, $k_L a$ is observed to be lower than in the mineral medium. As d_{32} and ε_G remain similar in broth-1 and broth-2, the decrease in $k_L a$ is attributed to a decrease in k_L (see Section 3.3), resulting in broth-2 and broth-3 having a similar $k_L a$ as water (without ethanol). Hence, the presence of biomass in these broths seems to diminish the beneficial effects of salts on $k_L a$. Supplemented ethanol causes a decrease in d_{32} , but the net increase in $k_L a$ is less pronounced than for the mineral medium. Still, the $k_L a$ values in the ethanol-rich broths are two to four times larger than the value in water without ethanol, indicating that the mass transfer properties of these broths can neither be represented by those of pure water, nor by those of water with added ethanol only.

In all cases with ethanol, a significant decrease in bubble diameter is observed ($p = 0.023$), as well as a narrower bubble size distribution by analyzing the standard deviations (Fig. 2b) and the bubble size distribution plots (Fig. S4). This confirms that ethanol stabilizes the homogeneous flow regime [32,44], while the coalescence inhibition causes a hold-up increase (Fig. 2c) [18].

In the mineral medium and broths, d_{32} does not change much, except

for some decrease with broth-3. This implies that the biomass, acetate and proteins have little effect on the bubble size at the observed concentrations. Broth-3 also shows a remarkably low d_{32} without ethanol, compared to the other mixtures. This effect cannot be explained, because more data are required to achieve correlation to the physical properties or the concentration of components.

The addition of ethanol significantly ($p = 0.0003$) increases the gas hold-up for all media (Fig. 2c). Increases in gas hold-up have been explained by coalescence inhibition: smaller bubbles rise slower, thereby increasing the gas residence time in the reactor and thus the hold-up [18]. The same applies for the mineral medium and broths: salts and surface-active compounds decrease d_{32} by inhibiting coalescence and thus increase the hold-up. For more details about the underlying mechanisms, one is referred to Keitel and Onken [17] and Jamialahmadi and Müller-Steinhagen [18].

The obtained data hint at a decrease in k_L due to supplemented ethanol (Fig. 2d), but this was not statistically significant ($p = 0.10$). A decrease would be explained by an ethanol layer causing extra mass transfer resistance around the gas bubble, or by increasing surface rigidity due to the small and spherical bubbles.

3.3. k_L as function of biomass concentration

From the different mixtures, it was observed that the fermentation broths have a lower k_L than water. This weakly correlates (Pearson's $r = -0.57$) with the biomass concentration in the broth (Fig. 3). It has been argued [9] that biomass increases broth viscosity and thus decreases k_L . However, the viscosities of the measured samples are in such a narrow range that a viscosity-based k_L model cannot adequately describe these changes [45] (Fig. S5). Any such reduction might be explained by a bubble surface blocking effect of the biomass, creating additional mass transfer resistance, even though direct oxygen consumption was not expected for syngas fermenting bacteria [9]. We expect that there are complex interactions between the (type of) microbe, salts and nutrients in the medium, and the products that influence the value of k_L . Unfortunately, at this moment, we are not able to provide general guidelines for prediction of k_L in fermentation broths without further experiments.

3.4. Comparison with empirical correlations

Empirical relations (Table S1) are often used for the prediction of $k_L a$ in bubble column fermentations [9]. After determining surface tension, density and viscosity for all the different media (with and without ethanol) (Table 1), the $k_L a$ values were calculated using these equations (Fig. 4). However, a large discrepancy is visible between the experimental and predicted values. These relations systematically underestimate $k_L a$ since they do not consider the influence of biomass, the salts and ethanol on the bubble properties. For example, the decrease in

Table 1
Physical properties of the different mixtures that were analyzed in this study. All properties were measured at 37 °C.

	Density	Viscosity	Surface tension	Ionic strength	Biomass concentration	Protein concentration	Acetic acid concentration	Ethanol concentration
	ρ_L kg m ⁻³	η_L mPa s	σ mN m ⁻¹	I mol L ⁻¹	C_x g L ⁻¹	C_{prot} g L ⁻¹	C_{Ac} g L ⁻¹	C_{EtOH} g L ⁻¹
Water	992.91	0.768	69.02	N/A	N/A	N/A	0	0.00
Mineral medium	996.28	0.768	68.62	0.296	N/A	N/A	0	0.00
Broth-1	997.97	0.821	70.43	0.296	0.061	0.369	1.14	0.00
Broth-2	997.62	0.732	68.82	0.171	0.246	1.278	2.22	0.18
Broth-3	996.60	0.763	69.08	0.136	0.087	0.062	1.37	0.12
Water + 5% ethanol	984.36	0.827	52.20	N/A	N/A	N/A	0	49.20
Mineral medium + 5% ethanol	986.63	0.825	51.49	0.277	N/A	N/A	0	49.20
Broth-1 + 5% ethanol	989.28	0.815	51.66	0.277	0.057	0.345	1.07	49.20
Broth-2 + 5% ethanol	988.06	0.840	51.83	0.160	0.230	1.196	2.07	49.37
Broth-3 + 5% ethanol	987.93	0.759	51.76	0.127	0.082	0.058	1.28	49.32

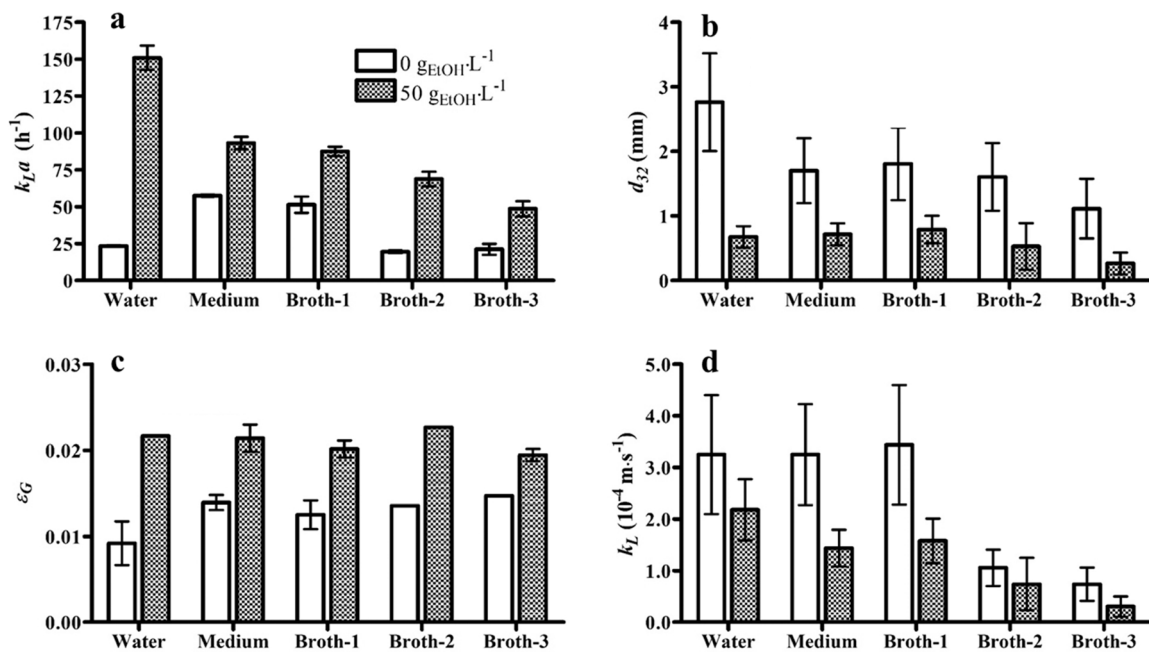


Fig. 2. Mass transfer characteristics obtained in a bubble column ($u_{G,s} = 1.8 \text{ mm s}^{-1}$) for different mixtures (see Table 1) without (white bars) and with supplemented ethanol (patterned bars). a) Volumetric mass transfer coefficient k_La , b) Sauter mean bubble diameter d_{32} , c) gas hold-up ϵ_G , and d) mass transfer coefficient k_L . Error bars: standard deviations between at least three measurements.

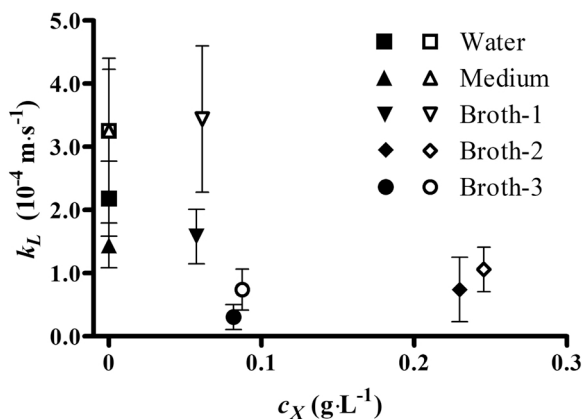


Fig. 3. Mass transfer coefficient k_L for different values of the biomass concentration in the studied mixtures. Open symbols: mixtures without ethanol, filled: mixtures with 50 g L^{-1} ethanol. Error bars: standard deviations.

surface tension by ethanol has a smaller influence on k_La in the empirical relations than observed in our experiments. As we saw that variables such as ionic strength, ethanol and biomass concentration are important regarding mass transfer in fermentation broths, these variables should also be part of such relationships.

3.5. Implications and future studies

This study shows several mass transfer characteristics obtained in different liquid mixtures relevant for gas fermentations. The obtained results show that the influence of medium components is significant and should be considered in future experimental and modeling work in gas fermentations. Our results are highly applicable to (industrial) practitioners of gas fermentation experiments as these can be beneficial for accurate determination of k_La and provides means to increase the k_La value by tuning medium composition.

Although it is widely known that antifoam promotes coalescence [9,

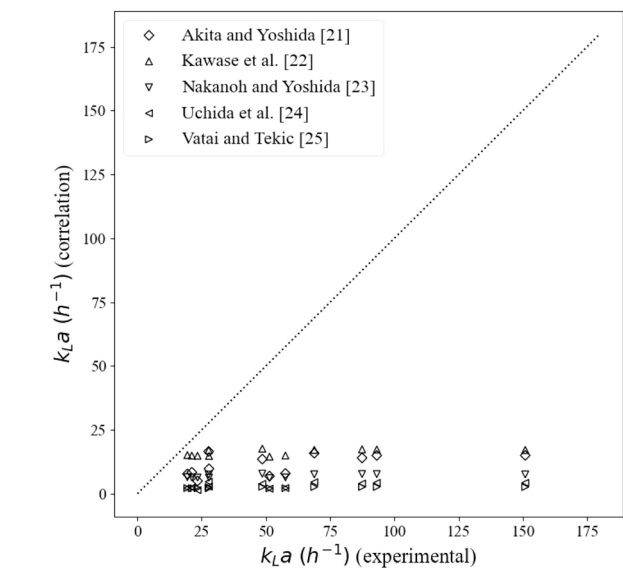


Fig. 4. Parity plot of experimental k_La data in the different mixtures vs. the values calculated using empirical relations [23–27].

15] and decreases mass transfer by creating a monolayer around the bubbles [46], and that dissolved solids (e.g., silica) can both improve and worsen mass transfer (depending on the concentration) [47], we did not consider their presence. Furthermore, only one mass transfer enhancing agent was studied (ethanol) for only one type of gas fermentation broths (derived from syngas) with a limited range of biomass concentrations ($0\text{--}0.5 \text{ g L}^{-1}$).

To reduce the redox potential, which is required for anaerobic (syngas) fermentation, a reducing agent was added to the fermentation broths (Table S2). Reaction of oxygen and the reducing agent (sodium sulfide) might have disturbed the dynamic absorption method for k_La determination (for broth-4), such that this broth had to be left out from the aforementioned evaluation. In such a case the method might be

adapted to, for example, the method proposed by Bandyopadhyay et al. [48]. Future studies should also note that the dynamic absorption method for $k_L a$ determination has low validity at high power inputs ($P/V > 1000 \text{ W m}^{-3}$) [49]. Considering that in air-water systems P/V is a critical variable determining the $k_L a$ value [9,50], further research should be done to determine the influence of P/V in cases with ethanol. Therefore the influence of variable superficial gas velocities (in BCRs and STRs) and stirrer speeds could be studied (STRs).

Industrial syngas fermentation requires higher biomass concentrations (around 10 g L^{-1}) [51] than the concentrations achieved in our experiments. Such high biomass concentrations are expected to influence the broth viscosity and thus the k_L . Furthermore, the used bubble column (7 cm diameter) is not representative for an industrial fermentation. To represent a large-scale bubble column, the column diameter should be more than 15 cm to exclude wall effects for mild viscous liquids [52]. For an industrial-scale syngas fermentation, a significantly higher gas flow velocity can be expected. We noted that at such gas velocities, determination of d_{32} in media without ethanol would be challenging due to the regime change to slug flow. To prevent slugs, we decided to compare d_{32} and $k_L a$ at low gas flow velocities. In literature, at higher gas flow rates and in wider and higher columns, the beneficial effect of ethanol on gas hold-up [32,44] and $k_L a$ has been observed [38]. Therefore, we think that the phenomena reported in this paper will also be present in large-scale reactors.

Our research indicates that other aspects of broth composition (next to ethanol content) influence mass transfer that is currently not well understood. Further research is needed to quantitatively predict the relevant parameters (e.g., k_L , d_{32}) in order to develop more realistic mass transfer models for fermentations. Although the exact mechanism might remain unknown, systematic experiments and technologies like machine learning might be used to develop algorithms for reliable prediction of mass transfer properties in fermentation broths of various compositions. Unraveling the mechanisms behind our observations (e.g., why $k_L a$ is lower in the mixture with mineral medium and ethanol compared to the water-ethanol mixture) will require additional and more fundamental studies, which could guide the development of mechanistic models.

Although our results were only obtained with supplemented ethanol, we stress that similar phenomena have been obtained with other compounds (longer alcohols, acids, ketones) [12,14,17]. This indicates that similar deviations in mass transfer characteristics can be expected in a wide range of gas fermentations [1], e.g., syngas fermentation (to alcohols/acids), microbial electrosynthesis (to acids) [7] as well as aerobic sugar-based fermentations (e.g., 1,4-butanediol production [53]). As there are methods available to measure mass transfer characteristics ($k_L a$, d_{32} , ϵ_G , k_L , a) easily, we highly recommend to perform these experiments with realistic broths to prevent underestimation of mass transfer rates.

4. Conclusions

By supplementing ethanol to water, $k_L a$ sharply and significantly increases, primarily by decreasing d_{32} . This effect is also present, but weaker, in the studied syngas fermentation broths. Broth salinity (ionic strength) and biomass concentration seem to affect $k_L a$ in fermentation broth as well. Future mass transfer studies should consider the influence of broth components because literature models fail to predict their effects.

CRedit authorship contribution statement

Lars Puiman: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing - review & editing. **Marina P. Elisiário:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Lilo M.L. Crasborn:** Conceptualization, Methodology, Investigation, Formal analysis. **Liselot E.C.H. Wagenaar:** Conceptualization, Methodology, Investigation. **Adrie J.J. Straathof:** Conceptualization,

Writing - review & editing, Supervision, Funding Acquisition. **Cees Haringa:** Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.bej.2022.108505](https://doi.org/10.1016/j.bej.2022.108505).

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