Study on mixing characteristics in shaken microwell systems

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Abstract

Shaken microwell plates are widely used for early bioprocess development as they allow a large number of experiments to be performed in parallel by using small amount of materials. Despite their widespread use, microwell plates have not been characterised from an engineering viewpoint. In this study, mixing time measurements were carried out in two wells of square and cylindrical cross sections for small orbital diameter shaker, d_o=3 mm, commonly used in commercial microwell platforms (i.e. ThermoMixer) and compared against measurements obtained in lab scale reactors for larger orbital diameters. The Dual Indicator System for Mixing Time (DISMT) method was employed for all the operating conditions investigated, and a range of rotational speeds was identified where mixing is less effective due to reduced free surface oscillation. An effective scaling parameter between microwell platforms and lab scale reactors was identified based on the natural frequency of the system, which depends only on fill volume, size and cross section of the reactor.

Highlights

- Reduced free surface oscillation leads to reduced mixing time in square microwell geometry.

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- Surface tension plays a critical role in the mixing process in square microwell plates.
- Mixing times are longer in cylinder wells at low shaken speeds when compared to square microwells.
- Natural frequency is a promising scaling parameter for microwells and larger reactors.

Keywords: Mixing, Orbitally shaken reactors, Mixing time, Microwells, Dual indicator method, Scaling

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1. Introduction

Bioreactors are used for growing organisms such as bacteria, yeast and mammalian cells under controlled operating conditions. For screening and process development studies at the lab scale, Orbitally Shaken Reactors (OSRs), such as Erlenmeyer flasks and microwell plates, are commonly employed. At larger scale, Stirred Tank Reactors (STRs) that rely on the use of stirrers to create correct fluid motion are the most common geometries. Due to the prevalence of STRs in the chemical and biochemical industries, several studies can be found in the literature that provide a thorough investigation of their mixing and fluid dynamics characteristics for a broad range of operating conditions [1, 2, 3, 4, 5, 6]. In recent years, the engineering characterisations of lab-scale OSRs have also been conducted by various researchers in terms of flow dynamics [7, 8, 9, 10, 11, 12], free surface motion [13, 14], solids suspension [15, 16] and power consumption [17, 18, 19], both experimentally and numerically.

Microscale technologies such as shaken microwell plates have been used extensively in early stage bioprocess development for screening and process optimisation, before the process can be implemented in conventional STRs. Unlike lab-scale OSRs which mainly comes in cylindrical geometry, microscale multi-well plates usually have square or cylindrical cross-sectional shapes and various bottom designs, such as flat, U-shaped or conical bottom. Most engineering characterisation studies in shaken microwell plates have focused on gas-liquid mass transfer [20, 21, 22, 23, 24] and power consumption [23, 25]. For example, Doig *et al* [22] experimentally measured the volumetric oxygen transfer coefficient (k_La) in three round microwells for a range of shaking diameters and frequencies, and developed a dimensionless numbers correlation. Dürauer *et al* [25] determined the local energy dissipation rate and power input in 6-, 24- and 96-well plates by adapting the clay/polymer flock system and demonstrated that the hydrodynamic stress is significantly different for various microwell plate formats.

In addition to k_La and power consumption studies, mixing time is also widely used to characterise macro-scale mixing performance in different types of bioreactors. Accurate mixing time determination in shaken reactors is critical to minimise concentration gradients that are deleterious to cell cultures. A technique based on two pH indicators was used by Tissot *et al* [26] to

measure mixing time in shaken cylindrical containers. The fastest mixing zone was found near the bottom, where mixing was mainly accomplished by diffusion. A single indicator system was used by Tan *et al* [27] to quantify mixing time in Erlenmeyer shaken flasks, and compared the results to those obtained in stirred tanks with different types of impellers. Contrary to the results obtained by Tissot et al. [26], they concluded that the orbital diameter does not significantly affect mixing time in shaken flasks. Rodriguez et al. [28] conducted mixing time measurements in OSRs and identified two mixing regimes, in-phase and out-of-phase, corresponding to the flow transition phenomenon reported by Weheliye et al. [9]. Their results indicated that mixing time in cylindrical reactors was also affected by feeding pipe position. For both regimes insertion was recommended close to the walls where wall boundary layer shear stresses are greater. Another study conducted by Rodriguez et al. [29] compared the mixing times obtained for shaken cylindrical reactors and Erlenmeyer flasks to those of STRs. The study suggested to use N/N_{cr} to account for various reactor sizes and fill volumes, rather than simply using N, as the ratio takes the presence of flow transition between in- or out-of-phase conditions into consideration. They also demonstrated that the Fr/Fr_{cr} is a useful parameter in scaling up/down between different reactor systems, where Fr_{cr} is the critical Froude number based on the rotational speed associated to the flow transition. Li *et al* [30] extended the mixing dynamics study to intermediate-sized OSRs for cylindrical and square geometries (d_i = 35.4 and 40.0 mm, respectively) shaken in the Eppendorf ThermoMixer C with a significantly reduced orbital diameter of 3 mm, in an effort to assess the validity of scaling law identified in [29] in smaller OSRs. The acceleration mode to achieve the final shaken speed in the cylindrical reactor was found to greatly affect the mixing time due to difference in free surface oscillations and a new scaling parameter based on the natural frequency of the reactor was suggested as it was demonstrated to be more effective for shaken systems with very small d_o.

A review of recent mixing time studies in shaken reactors is summarised in Table 1. It is evident in Table 1 that mixing time quantification has been conducted by a number of researchers, however, the vast majority of them focused on Erlenmeyer flasks and lab-scale cylindrical reactors. Despite the wide use of microwell plates in bioprocess development, the mixing process in microwell plates was only considered by two studies [31, 23] more than a decade ago and primarily by means of CFD and intrusive experimental tech-

nique. The current work aims at extending the DISMT technique established for larger OSRs further down to microwell systems of different geometries for reduced shaking orbital diameter (d_o =3 mm) and to assess the effects of fluid properties (viscosity and surface tension) on mixing time. It builds upon previous studies of Rodriguez *et al* [29] and Li *et al* [30] and aims at providing a thorough understanding of the mixing process induced by orbital shaking motion in microwell plates. From this perspective, mixing time was measured in two microwells (d_i =17.1 and 15.6 mm, respectively) with square and cylindrical geometries, which are mimics of commercially-available microtitre plates, by means of DISMT technique.

2. Materials and methods

2.1. Microwell plates and equipment

Two single well mimics made of acrylic plastic were used, having the same geometry of one well from commercial 24 deep square well (24 DSW) and 24 standard round well (24 SRW) plates, purposely made by the UCL Department of Chemical Engineering Workshop. The height of the cylindrical well, h_v, was increased from 17 mm (height of the original well in 24 SRW) to 44 mm to allow higher fluid volume to be assessed. The impacts of three parameters on mixing time were investigated in the square well, including fluid volume, V_f, fluid viscosities of 10 and 100 times greater than that of water (v/v_w = 10 and 100, respectively), and surface tension in the range of 31.8 to 68.4 mNm^{-1}. Three fill volumes (V_f = 3, 4 and 5 mL) were used to measure mixing time in the cylindrical well and the results were compared to the square well to assess the effect of microwell geometry on mixing performance. The full geometrical details of the two wells and operating conditions investigated in this work are summarised in Table 2.

The experimental set up is shown in Figure 1, where measurements were made using an Eppendorf ThermoMixer C, with a fixed orbital diameter of 3 mm. In the set up, different wells can be screwed into a plate, which is placed in the plate holder on the ThermoMixer. An extension arm was built on the plate to allow the synchronisation of the movement of the NET iCube camera and the shaker. An LED panel was fixed at the back of the plate to reduce background noises and provide uniform illumination. A 20 µL Gilson pipette, attached to a clamp stand, was used for the addition of base to initiate the chemical reaction. The clamp stand ensured the feeding

location and height were consistent for each experiment. The rotational speed investigated ranged from 300 to 1000 rpm in increments of 50 rpm.

2.2. Surface tension measurement

The surface tension of different solutions in the square well was determined using the Wilhelmy plate principle which is considered as one of the most appropriate ring method for surface tension measurement, due to the fact that no hydrostatic correction is required. The digital KRÜSS K100 Force Tensiometer and a platinum plate (20 × 10 × 0.1 mm) were used to obtain surface tension measurement. The solution was placed in a thermostated flask at 25°C and temperature was constantly monitored by a thermostat. Three repetitions were carried out for each condition with the average value being recorded. An anionic surfactant sodium dodecyl sulphate (SDS) supplied by Sigma Aldrich, was dissolved in de-ionised water and the desired solution concentration was obtained by successive dilutions. The initial SDS concentration in the solution was 3.47×10^{-3} molL^{-1} and diluted by factors of 10, 50 and 100. The surfactant concentrations and dynamic surface tension measured by the tensiometer are shown in Table 3.

2.3. DISMT technique

The technique used to estimate mixing time in microscale systems was adapted from the method developed by Melton et al. [32], namely Dual Indicator System for Mixing Time. As the name implies, two acid-base pH indicators - methyl red and thymol blue, were present in the bulk solution, which allow a direct visualisation of pH change, with fluid being red, blue and yellow for pH values less than 6.3, greater than 8 and between 6.3 and 8, respectively. The colour change of the solution was initiated by adding small amount of sodium hydroxide, where the base rapidly react with the hydrochloric acid that is already present in the bulk solution. The colour change of the solution only occur when reactants have been mixed at the molecular level for such a reaction-based technique, thus avoiding the "micromixing" phenomenon associated with dilution-based techniques. The capture speed of the iCube camera is 16 frames per second, therefore allowing short mixing time, even less than 0.1 second, to be effectively determined.

The stock solution of pH indicators were prepared in 70% ethanol and the working reagent for DISMT test was prepared in de-ionised water containing thymol blue (Fisher Scientific) and methyl red (Fisher Scientific) solutions,

with percent concentrations of 4.67 $\times$ 10^{-3} v/v and 4.26 $\times$ 10^{-3} v/v, respectively. Sodium hydroxide was subsequently added to the well-mixed red solution in increment of 2 µL until a bright yellow colour was obtained. The desired volume of DISMT solution was measured into the well and it was acidified initially by adding small amount of 0.075 M HCl. The solution was red at the beginning and the reaction between acid and base was initiated by the insertion of the same amount of 0.075 M NaOH after the flow in the well was fully established. Each condition was repeated ten times to reduce the statistical error and the average of the ten measurements were used as the mixing time. The working fluid could be re-used up to three times before replacing it with a fresh solution. To prepare solution with higher viscosity, absolute glycerol was mixed with certain amount of de-ionised water to obtain desired fluid viscosity. The viscosity of the glycerol/water aqueous solution was determined based on the method proposed by Cheng [33]. The image processing method used in this work was the same as that used by Rodriguez et al. [29] and Li et al. [30] who measured mixing time in larger OSRs, thus allowing a more reliable and objective comparison of mixing time across different reactor scales.

3. Results and discussion

3.1. Mixing time in 24-DSW plate format

The impact of three parameters, namely fill volume, fluid viscosity and surface tension, on mixing time were investigated in the square well, a mimic of the 24-DSW plate, while shaken on the ThermoMixer C with d_o = 3 mm.

Figure 2 shows the mixing time variation, t_m, with respect to shaken speeds for five different fill volumes, ranging from 2 to 6 mL. It should be noted that the log scale of t_m was used to allow better visualisation of the impact of fill volume on mixing time. A significant mixing time reduction (from 100 s to 10 s) occurred in the lower end range of shaken speeds investigated, between N = 300 - 400 rpm. It is also evident that the mixing time for V_f = 6 mL was approximately 3.5 times greater than that of 2 mL at the lowest speed. For shaken speeds greater than 400 rpm, the mixing process became very rapid with mixing time less than 10 seconds for all fill volumes examined. At higher speed range, it is evident that an unexpected increase in mixing time occurred at N = 550 - 650 rpm for V_f ranging from 3 to 6 mL, which was more pronounced the larger the fill volume. This phenomenon occurred

earlier at $N = 500$ rpm for the smallest fill volume investigated. Mixing time dropped again with further increase in shaken speed and no significant change was noticed afterwards.

The unexpected mixing time increase at high speed range was associated with a change in the free surface dynamics in the well and most likely results from the very small orbital shaken diameter used. To further understand this unexpected phenomenon, the non-dimensional wave amplitude, $\Delta h/d_i$, of the free surface was measured, where Δh is the difference between the highest and lowest fluid height at the centre of the reactor. Figure 3 displays both the t_m variation (left y-axis) and the $\Delta h/d_i$ variation (right-axis) with increasing shaken speed in the range of 450 to 800 rpm where the unexpected mixing time increase occurred, for $V_f = 6$ mL. It is evident that an increase in mixing time was resulted from a decrease in free surface height and vice versa. A similar phenomenon was also noticed in the work of Li et al. [30] where a direct acceleration mode affected the free surface oscillations and thus the mixing time in an intermediate-sized cylindrical reactor when shaken in ThermoMixer. The reduced free surface oscillation in the square microwell is independent of acceleration modes, however, the longer mixing time at high speed range was also due to a significantly reduced free surface height.

To further elucidate the cause of the reduced free surface oscillation, the non-dimensional wave amplitude was also measured in the square well for the same fill volume (6 mL) when shaken on a lab shaker with $d_o = 15$ mm. The results were compared to those obtained for $d_o = 3$ mm in Figure 4 where $\Delta h/d_i$ was plotted against Fr instead of N, in order to make the two sets of results more comparable. Consistently with Figure 2, when shaken at $d_o = 3$ mm, the non-dimensional wave amplitude dropped when Fr was increased to 0.6 corresponding to 600 rpm, which is the speed for the occurrence of the mixing time increase. On the contrary, the wave amplitude exhibited a constant increase with shaken speed when a larger d_o was employed. Therefore, the unexpected mixing time increase in the square well has to be related to the small orbital diameter used which has a significant impact on the free surface oscillation.

Cell culture media used in bioprocesses generally have a viscosity similar to water, however, higher viscosities might be encountered in serum-based me-

dia for cell expansion process or in high cell density cultures. The viscosity of cell culture media in the latter context increases with time due to the presence of increasing amount of biomass. To this perspective, mixing time measurements by DISMT technique were also carried out in the square well for two fluids exhibiting greater viscosity than water and the mixing time variation with increasing shaken speed is illustrated in Figure 5. For the fluid with viscosity of 10^{-4} m^2s^{-1} (100 times more viscous than water) at low shaken speed range, the mixing time showed approximately a 7-fold increase as shown in Figure 5. The fluid with viscosity that is 10 times greater than water also exhibited an increase in mixing time at 300 - 350 rpm but to a much lesser extent compared to the previous discussed case. At higher speed range, it is evident that the increase in mixing time also occurred for viscous fluids and was the extent was even more pronounced. It is also worth noting that the speeds at which t_m increases for water, fluid with 10× viscosity and fluid with 100× viscosity are 600, 650 and 700 rpm, respectively. This indicate that this mixing time increase phenomenon occurs at a higher shaken speed, the more viscous the fluid is. For viscous fluids, the increase in t_m at high speed range is also resulted from a reduced free surface oscillation.

Additional mixing time experiments were performed to assess the effect of surface tension on mixing dynamics in the square microwell as the interficial tension of a liquid in the well is influenced by the gas phase and the physiochemical property of the liquid, known as the gas-liquid interface. SDS, an anionic surfactant, was added into the bulk solution and successive dilutions were performed to obtain liquid with different surface tensions. Four surfactant concentrations were investigated and the results were compared to pure water when the square well was filled with 3 mL of liquid. As depicted in Figure 6, the mixing time decreased with increasing surfactant concentrations when shaken at the lowest speed (300 rpm). It is also evident that in the presence of surfactant the unexpected increase of mixing time at high speed range, N = 600 - 650 rpm, reduced by a great extent. Therefore, the reduction of surface tension with the addition of surfactant has a significant influence on the mixing dynamics in the square microwell, both at low and high shaken speeds, suggesting surface tension is a key factor that needs to be considered during orbital shaking motions, especially at small orbital diameters. It is also worth noting that the properties of the well construction material influences the liquid-solid interface, thus also impacting on the

surface tension as indicated in the Young equation:

$$\sigma_{V/S} = \sigma_{L/V}(cos\theta) + \sigma_{L/S} \quad (1)$$

Where $\sigma_{V/S}$, $\sigma_{L/V}$ and $\sigma_{L/S}$ are vapour-solid, liquid-vapour and liquid-solid interficial tensions, respectively, and θ is the contact angle between the solid and liquid phases. As suggested in Equation (1), the construction material of the microwell needs to be taken into account when assessing the effect of surface tension on mixing.

3.2. Comparison of mixing time in different well geometries

Microwell plates with cylindrical geometry, such as the 24-SRW plate, are also commonly used for screening and process development studies. In this section, mixing time was measured in the cylindrical microwell for three different fill volumes (V_f = 3, 4 and 5 mL) and the results were compared with those obtained in the square well with similar dimensions to assess the effect of reactor geometry on mixing dynamics in shaken microwell systems.

At low shaken speeds, the mixing process is longer in the cylindrical well than the square one when the same fill volume is considered, as shown in Figure 7. The mixing process is more efficient in the square well, most likely due to the presence of the four corners of a square well, as they acting similarly as baffles which promoting the mixing performance in bioreactors. Similarly in the cylindrical well, mixing time dropped significantly with increasing shaken speed in the low speed range (i.e. 300 - 400 rpm), while no significant change in mixing time observed for shaken speeds greater than 400 rpm and the mixing process became very rapid afterwards. It should also be noticed that contrary to the work of Li et al. [30], no difference in mixing time was identified in the cylindrical microwell when different acceleration modes was adopted.

Nevertheless, the phenomenon of small increase in mixing time in the high speed range was not observed in the cylindrical geometry as shown in the close up of Figure 8a. The deformation of the free surface is the most visible effect of orbital shaking motion and the liquid flow within a container is uniquely defined by the pattern of its free surface, thus the free surface shape was captured by the high speed camera to allow comparison between the two geometries in terms of free surface dynamics. As shown in the free

surface snapshots for the two geometries in Figure 8b, when the cylindrical well is considered, it is evident that the free surface inclination, and therefore oscillation, continuously increases with increasing speed, resulting in faster mixing. However, when the square geometry is considered, it is clear that at speeds of 650 and 700 rpm, the free surface does not show a pronounced inclination, indicating that the sloshing mechanism and, therefore the mixing process, is reduced. It is evident from the observations that the mixing process in shaken microwell systems is in close association with the free surface dynamics.

3.3. OSR scaling based on Froude number

The mixing time measurements obtained in the two microwells as presented in Section 3.1 and 3.2, are compared to those obtained in larger OSRs (d_i = 8 - 13 cm) and greater orbital diameters (d_o = 1.5 - 5 cm) as previously reported by Rodriguez et al. [29]. The mixing time results between current work and [29] are comparable as the same experimental methodology and image processing algorithms were used. The comparison between different results are in an attempt to derive a more universal scaling law for OSRs with various scales, geometries and operating conditions based on the mixing dynamics within the reactors.

In the study of Rodriguez et al. [29], two dimensionless parameters, mixing number (Nt_m) and the ratio of Fr/Fr_{cr}, were identified as effective scaling parameters for mixing time measurements obtained in their work and those presented by Tissot et al. [26] for very different reactor sizes and operating conditions. They proposed a power law relationship for scaling in the form of

$$Nt_m = a\left(\frac{Fr}{Fr_{cr}}\right)^{-b} + c \tag{2}$$

Where a, b and c are three constants accounting for different reactor configurations. They found values of a, b and c of 100.7, -1.245 and 25, respectively, best fit their data with a wide range of operating conditions. The use of the critical Froude number in scaling was found to be more effective than simply using Fr, as Fr_{cr} takes the presence of flow transition between in- and out-of-phase in the OSR into account, as described in Weheliye et al. [9].

To assess the possibility of extending the scaling law identified in Rodriguez et al. [29] to microwell systems, their results obtained in larger cylindrical

reactors for five parameter combinations and the proposed power law function are plotted in Figure 9 and compared against the mixing number curves for the two microwells with square and cylindrical geometries. It is evident on Figure 9 that the model derived by Rodriguez et al. [29] tends to overpredict the Nt_m trend obtained in microwell systems, most likely due to the small orbital diameter employed that led to different fluid and mixing dynamics within the wells. Therefore, there is a need to identify another parameter to aid the scaling across different shaken systems.

3.4. OSR scaling based on natural frequency

A potential scaling parameter was identified in the work of Reclari et al. [14] who demonstrated that the natural frequency of a shaken cylindrical container is directly related to the free surface oscillations. As described in Ibrahim [34], depending on the geometry of the reactor, the natural frequency could be calculated from Equation Equation (3) for upright cylinders and from Equations Equation (4) and Equation (5) for rectangular containers. It is evident from its definitions that the natural frequency only depends on the reactor size and amount of fluid inside, but independent of shaken orbital diameter.

$$\omega_{11}^2 = \frac{2G\epsilon_{11}}{d_i} \tanh\left(\frac{2\epsilon_{11}h_f}{d_i}\right) \tag{3}$$

$$\omega_{11}^2 = Gk_{mn} \tanh(k_{mn}h_f) \tag{4}$$

$$k_{11} = \pi\sqrt{\frac{8}{d_i}} \tag{5}$$

Where $\epsilon_{11} = 1.841$ and it is the first root of the derivative of the Bessel's function of the first kind and first order.

Mixing number variation for the two microwells are plotted together with those obtained in Rodriguez et al. [29] for five conditions (three reactor sizes and three fluid heights in one reactor) in Figure 10 based on the ratio of angular velocity and natural frequency ω/ω_{11}. It is evident that the two datasets correlate better by using ω/ω_{11} as the scaling parameter rather than Fr/Fr_{cr}, most likely due to the independence of orbital diameter of natural frequency. The natural frequency was also identified as an effective parameter in Li et al. [30] who considered mixing dynamics in intermediate-sized reactors shaken at $d_o = 3$ mm. It could therefore be concluded that

ω/ω_{11} acts as a better scaling parameter for OSRs if microwell plates and reduced orbital diameter are used.

4. Conclusions

In this study, the DISMT technique for mixing time measurement has successfully been applied to microwell systems shaken with significantly reduced orbital diameter. The effects of fill volume, fluid viscosity and surface tension on mixing time were investigated in 24 DSW and the difference of mixing dynamics between square and cylindrical geometries were assessed in a ThermoMixer with $d_o = 3$ mm. The mixing time of the DSW showed in general the typical variation of a mixing number curve, however it was identified a range of shaken speed $N = 600$ - 650 rpm, which was denoted by an increased of mixing time with speed. This phenomenon is caused by a reduced free surface oscillation over this range of speeds, which does not occur when a cylindrical geometry is considered. With a reduction of surface tension this phenomenon disappears also in the deep square well. Mixing time measurements obtained in this work were compared to those reported previously in lab-scale shaken reactors by Rodriguez et al. [29]. These data indicate that the natural frequency can be used as an effective parameter to scale between microwells and larger scale shaken reactors, mostly due to the independence of orbital diameter of natural frequency.

[1] Z. Doulgerakis, M. Yianneskis, A. Ducci, On the interaction of trailing and macro-instability vortices in a stirred vessel-enhanced energy levels and improved mixing potential, Chemical Engineering Research and Design 87 (2009) 412–420.

[2] Z. Doulgerakis, M. Yianneskis, A. Ducci, On the manifestation and nature of macroinstabilities in stirred vessels, AIChE Journal 57 (2011) 2941–2954.

[3] A. Ducci, M. Yianneskis, Direct determination of energy dissipation in stirred vessels with two-point lda, AIChE Journal 51 (2005) 2133–2149.

[4] A. Ducci, M. Yianneskis, Vortex tracking and mixing enhancement in stirred processes, AIChE Journal 53 (2007) 305–315.

[5] R. Escudié, A. Liné, Experimental analysis of hydrodynamics in a radially agitated tank, AIChE Journal 49 (2003) 585–603.

[6] M. Yianneskis, Z. Popiolek, J. Whitelaw, An experimental study of the steady and unsteady flow characteristics of stirred reactors, Journal of Fluid Mechanics 175 (1987) 537–555.

[7] J. Gardner, G. Tatterson, Characterization of mixing in shaker table containers, Biotechnology and Bioengineering 39 (1992) 794–797.

[8] H. M. Kim, J. P. Kizito, Stirring free surface flows due to horizontal circulatory oscillation of a partially filled container, Chemical Engineering Communications 196 (2009) 1300–1321.

[9] W. Weheliye, M. Yianneskis, A. Ducci, On the fluid dynamics of shaken bioreactors—flow characterization and transition, AIChE Journal 59 (2013) 334–344.

[10] A. Ducci, W. H. Weheliye, Orbitally shaken bioreactors—viscosity effects on flow characteristics, AIChE Journal 60 (2014) 3951–3968.

[11] E. Mancilla, C. Palacios-Morales, M. Córdova-Aguilar, M. Trujillo-Roldán, G. Ascanio, R. Zenit, A hydrodynamic description of the flow behavior in shaken flasks, Biochemical Engineering Journal 99 (2015) 61–66.

[12] W. H. Weheliye, N. Cagney, G. Rodriguez, M. Micheletti, A. Ducci, Mode decomposition and lagrangian structures of the flow dynamics in orbitally shaken bioreactors, Physics of Fluids 30 (2018) 033603.

[13] M. Discacciati, D. Hacker, A. Quarteroni, S. Quinodoz, S. Tissot, F. M. Wurm, Numerical simulation of orbitally shaken viscous fluids with free surface, International Journal for Numerical Methods in Fluids 71 (2013) 294–315.

[14] M. Reclari, M. Dreyer, S. Tissot, D. Obreschkow, F. M. Wurm, M. Farhat, Surface wave dynamics in orbital shaken cylindrical containers, Physics of Fluids 26 (2014) 052104.

[15] E. Olmos, K. Loubiere, C. Martin, G. Delaplace, A. Marc, Critical agitation for microcarrier suspension in orbital shaken bioreactors: Experimental study and dimensional analysis, Chemical Engineering Science 122 (2015) 545–554.

[16] I. Pieralisi, G. Rodriguez, M. Micheletti, A. Paglianti, A. Ducci, Microcarriers' suspension and flow dynamics in orbitally shaken bioreactors, Chemical Engineering Research and Design 108 (2016) 198–209.

[17] J. Büchs, U. Maier, C. Milbradt, B. Zoels, Power consumption in shaking flasks on rotary shaking machines: I. power consumption measurement in unbaffled flasks at low liquid viscosity, Biotechnology and Bioengineering 68 (2000) 589–593.

[18] J. Büchs, S. Lotter, C. Milbradt, Out-of-phase operating conditions, a hitherto unknown phenomenon in shaking bioreactors, Biochemical Engineering Journal 7 (2001) 135–141.

[19] W. Klöckner, J. Büchs, Advances in shaking technologies, Trends in Biotechnology 30 (2012) 307–314.

[20] R. Hermann, M. Lehmann, J. Büchs, Characterization of gas–liquid mass transfer phenomena in microtiter plates, Biotechnology and Bioengineering 81 (2003) 178–186.

[21] W. A. Duetz, B. Witholt, Oxygen transfer by orbital shaking of square vessels and deepwell microtiter plates of various dimensions, Biochemical Engineering Journal 17 (2004) 181–185.

[22] S. D. Doig, S. C. Pickering, G. J. Lye, F. Baganz, Modelling surface aeration rates in shaken microtitre plates using dimensionless groups, Chemical Engineering Science 60 (2005) 2741–2750.

[23] H. Zhang, S. R. Lamping, S. C. Pickering, G. J. Lye, P. A. Shamlou, Engineering characterisation of a single well from 24-well and 96-well microtitre plates, Biochemical Engineering Journal 40 (2008) 138–149.

[24] M. Funke, S. Diederichs, F. Kensy, C. Müller, J. Büchs, The baffled microtiter plate: increased oxygen transfer and improved online monitoring in small scale fermentations, Biotechnology and Bioengineering 103 (2009) 1118–1128.

[25] A. Dürauer, S. Hobiger, C. Walther, A. Jungbauer, Mixing at the microscale: Power input in shaken microtiter plates, Biotechnology Journal 11 (2016) 1539–1549.

[26] S. Tissot, M. Farhat, D. L. Hacker, T. Anderlei, M. Kühner, C. Comninellis, F. Wurm, Determination of a scale-up factor from mixing time studies in orbitally shaken bioreactors, Biochemical Engineering Journal 52 (2010) 181–186.

[27] R.-K. Tan, W. Eberhard, J. Büchs, Measurement and characterization of mixing time in shake flasks, Chemical Engineering Science 66 (2011) 440–447.

[28] G. Rodriguez, W. Weheliye, T. Anderlei, M. Micheletti, M. Yianneskis, A. Ducci, Mixing time and kinetic energy measurements in a shaken cylindrical bioreactor, Chemical Engineering Research and Design 91 (2013) 2084–2097.

[29] G. Rodriguez, T. Anderlei, M. Micheletti, M. Yianneskis, A. Ducci, On the measurement and scaling of mixing time in orbitally shaken bioreactors, Biochemical Engineering Journal 82 (2014) 10–21.

[30] Y. Li, A. Ducci, M. Micheletti, Mixing time in intermediate-sized orbitally shaken reactors with small orbital diameters, Chemical Engineering & Technology 42 (2019) 1611–1617.

[31] S. Weiss, G. T. John, I. Klimant, E. Heinzle, Modeling of mixing in 96-well microplates observed with fluorescence indicators, Biotechnology Progress 18 (2002) 821–830.

[32] L. A. Melton, C. Lipp, R. Spradling, K. Paulson, Dismt-determination of mixing time through color changes, Chemical Engineering Communications 189 (2002) 322–338.

[33] N.-S. Cheng, Formula for the viscosity of a glycerol- water mixture, Industrial & Engineering Chemistry Research 47 (2008) 3285–3288.

[34] R. A. Ibrahim, Liquid sloshing dynamics: theory and applications, Cambridge University Press, 2005.

[35] D. F. Gerson, M. M. Kole, Quantitative measurements of mixing intensity in shake-flasks and stirred tank reactors: use of the mixmeter, a mixing process analyzer, Biochemical Engineering Journal 7 (2001) 153–156.

[36] H. Zhang, W. Williams-Dalson, E. Keshavarz-Moore, P. A. Shamlou, Computational-fluid-dynamics (cfd) analysis of mixing and gas–liquid mass transfer in shake flasks, Biotechnology and Applied Biochemistry 41 (2005) 1–8.

[37] G. Rodriguez, M. Micheletti, A. Ducci, Macro-and micro-scale mixing in a shaken bioreactor for fluids of high viscosity, Chemical Engineering Research and Design 132 (2018) 890–901.

Table 1: Summary of mixing time measurements carried out in shaken systems, ordered from the largest to the smallest reactor size.

	Ref.	Reactor geometry and size	Operating conditions	Measurement technique
Lab-scale	Rodriguez et al (2014) [29]	Three cylindrical vessels with di = 70, 100 and 130 mm and two Erlenmeyer flasks with nominal volume of 1 and 2 L	do = 25 mm, N = 90 - 210 rpm	DISMT technique
	Gerson & Kole (2001) [35]	1 L Erlenmeyer flask	N = 0 - 200 rpm, V_f = 540 mL	Mixmeter probe
	Tan et al (2011) [27]	Three Erlenmeyer flasks with nominal volumes of 100, 250 and 500 mL	d_o = 25 - 50 mm, N = 100 - 350 rpm, V_f = 10% of the nominal volume	Single pH indicator colourimetric technique
	Zhang et al (2005) [36]	250 mL Erlenmeyer flask	d_o = 20 - 60 mm, N = 100 - 300 rpm, V_f = 25 - 100 mL	CFD simulation
	Tissot et al (2010) [26]	Cylindrical vessel with d_i = 10, 13, 16, 28.7 and 125 cm	Various d_o, N and V_f	DISMT technique
	Rodriguez et al (2013) [28]	Cylindrical vessel with d_i = 100 mm	d_o = 25 mm, N = 60 - 140 rpm, V_f = 235 - 550 mL	DISMT technique
	Rodriguez et al (2018) [37]	Cylindrical vessel with d_i = 100 mm	d_o = 25 mm, N = 100 - 200 rpm, three viscosities	DISMT technique
	Li et al (2019) [30]	A cylindrical with d_i = 40 mm and a square vessel with side = 35.4 mm	d_o = 3, 15 and 50 mm, N = 100 - 1000 rpm, h/d_i = 0.5, 0.75 and 1	DISMT technique
Micro-scale	Zhang et al (2008) [23]	24- and 96-deep square well with d_i = 17 and 8 mm, respectively	d_o = 3 mm, N = 500 - 1500 rpm	CFD simulation
	Weiss et al (2002) [31]	96-well with either round or flat bottom	d_o = 12 mm, N = 500 - 900 rpm, V_f = 200 μL	Fluorescence pH sensor and soluble pH indicators

Table 2: Geometrical characteristics of the two well mimics and list of operating conditions investigated

	Square well with conical bottom	**Cylindrical well with flat bottom**
Internal Diameter d_i [mm]	17.1	15.6
Fluid height h_f [mm]	6.9 - 20.8	15.7 - 26.2
Fill volume V_f [mL]	2 - 6	3 - 5
Fluid viscosity $[m^2 s^{-1}]$	10^{-4}, 10^{-5}, 10^{-6}	\

Table 3: Surfactant concentrations and values of surface tension measured by the tensiometer

Surfactant concentration molL^{-1}	**Dilution factor**	**Dynamic surface tension mNm^{-1}**
0	\	72.3
3.47×10^{-3}	0	68.4
3.47×10^{-4}	10	51.1
6.94×10^{-5}	50	44.9
3.47×10^{-5}	100	31.8

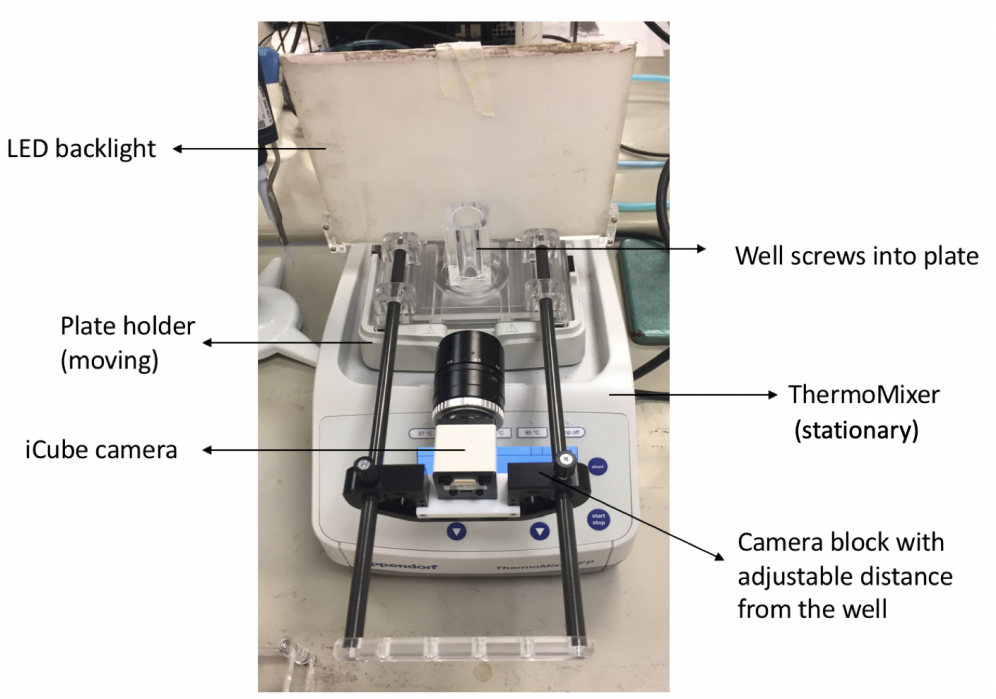


Figure 1: Set up for DISMT experiments on Eppendorf ThermoMixer C.

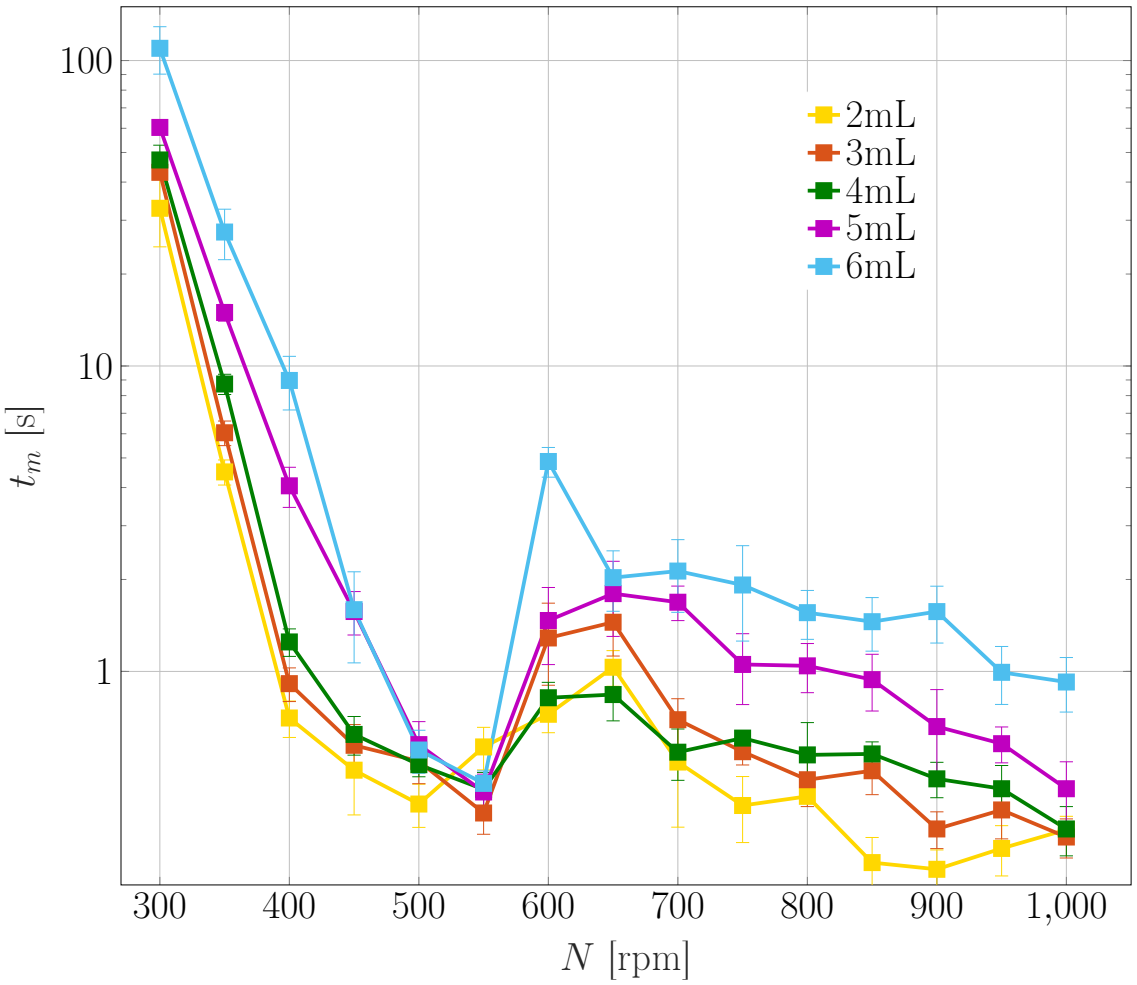


Figure 2: The effect of fill volume on mixing time in the 24-DSW format.

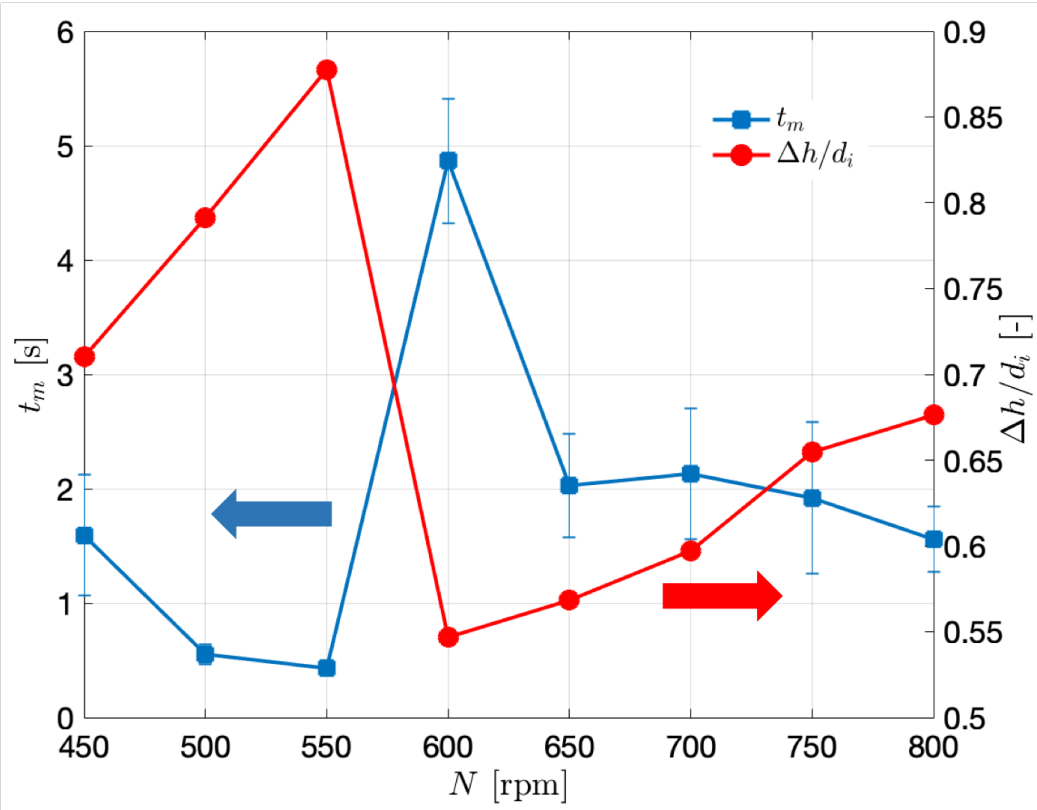


Figure 3: Comparison between mixing time and non-dimensional free surface amplitude in the square well with $V_f = 6$ mL.

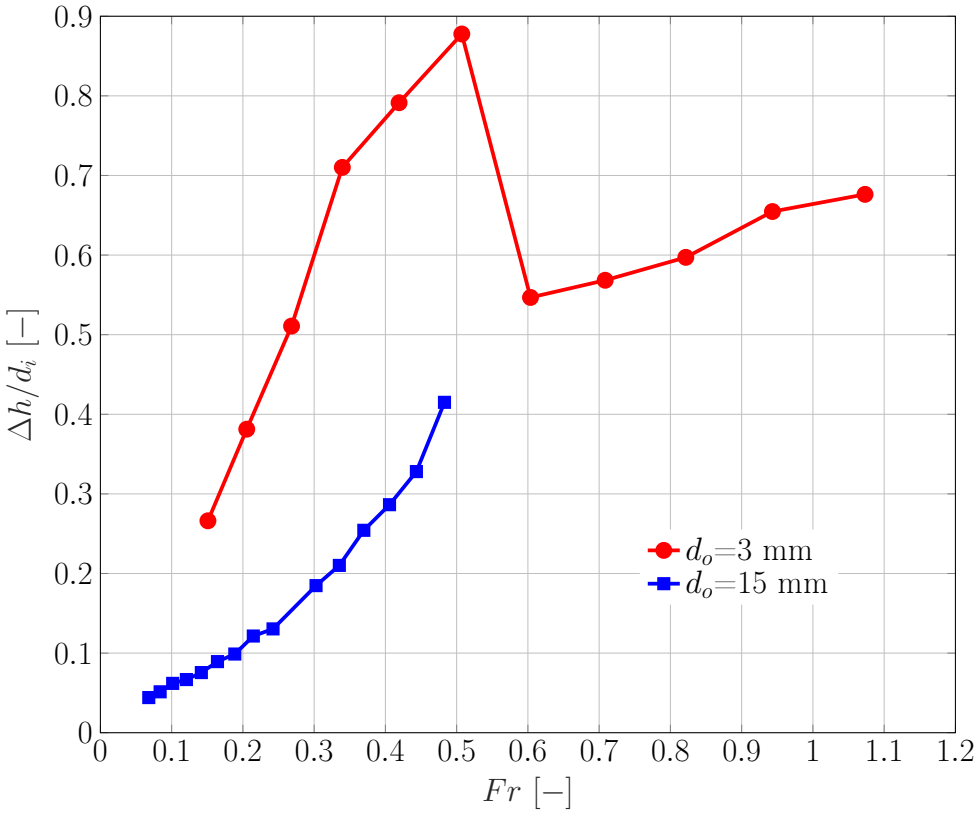


Figure 4: Non-dimensional free surface amplitude versus Froude number for two different orbital diameters in the square well with $V_f = 6$ mL .

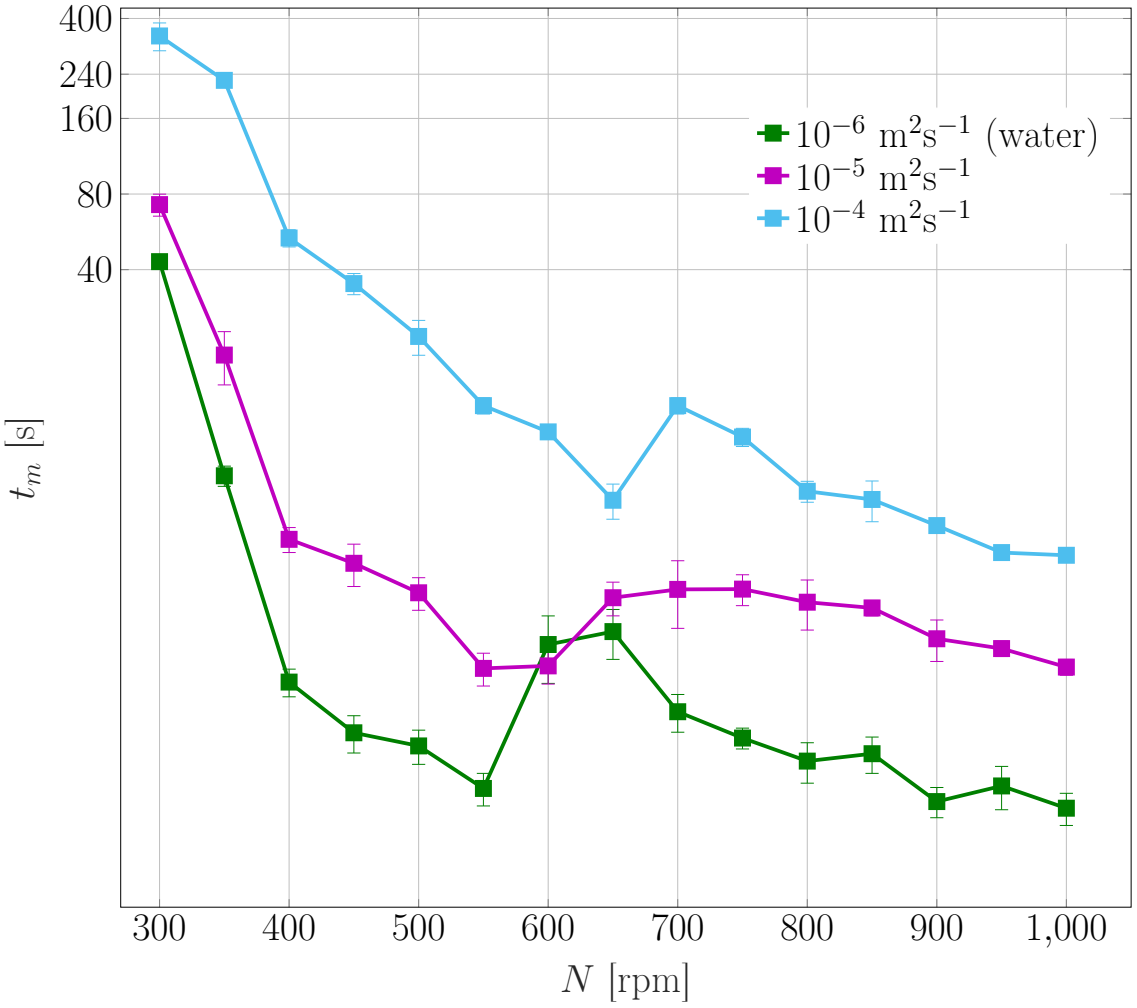


Figure 5: The effect of fluid viscosity on mixing time in the 24-DSW format with V_f=3 mL.

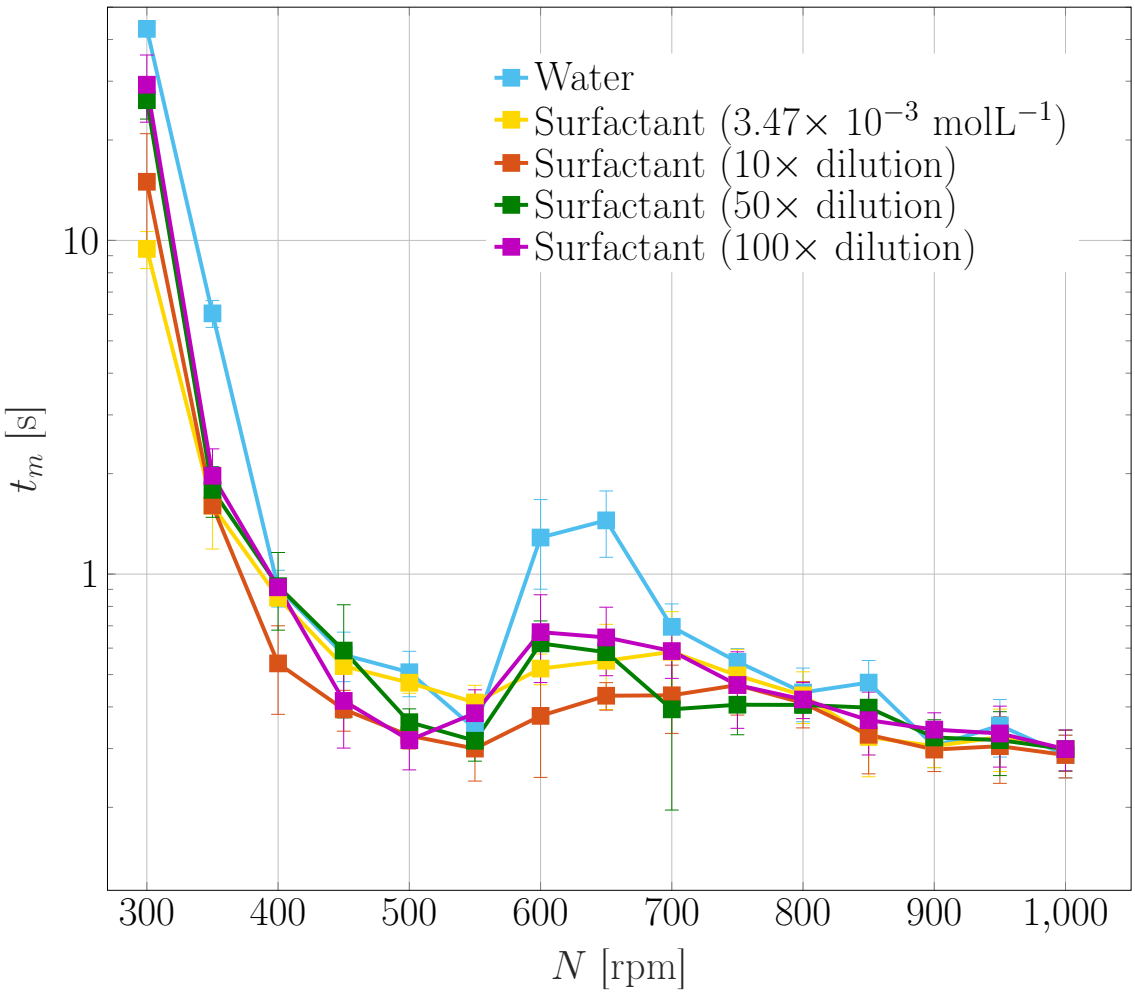


Figure 6: The effect of surface tension on mixing time in the 24-DSW format with V_f=3 mL.

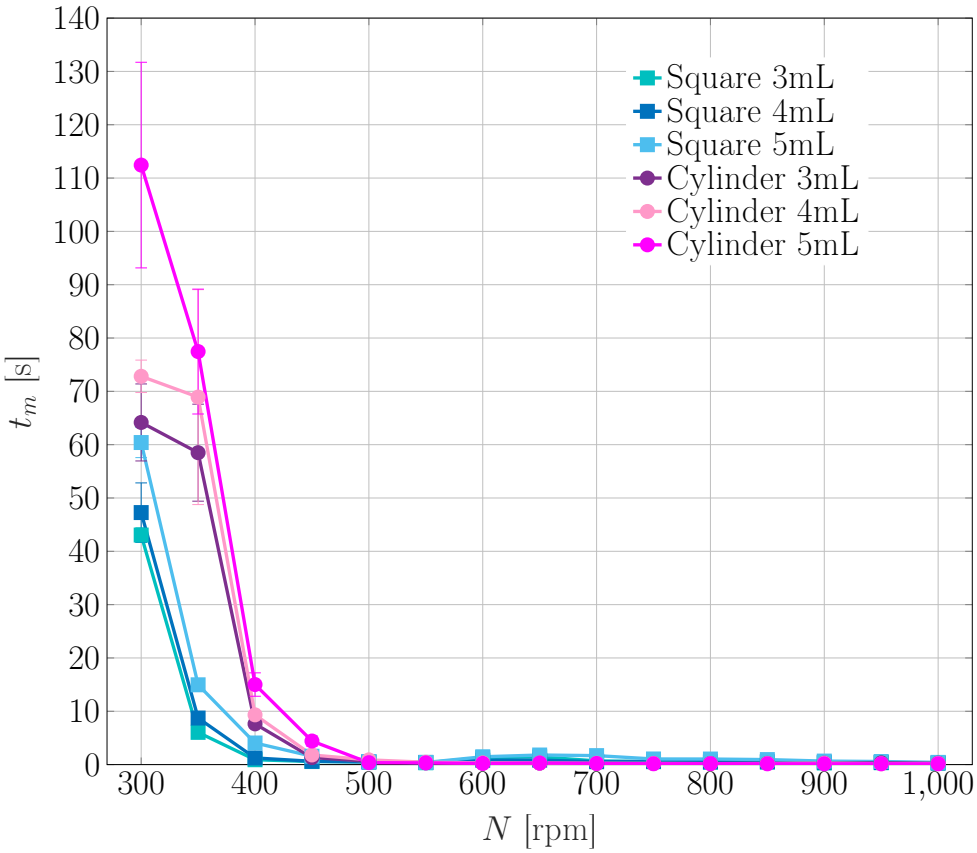
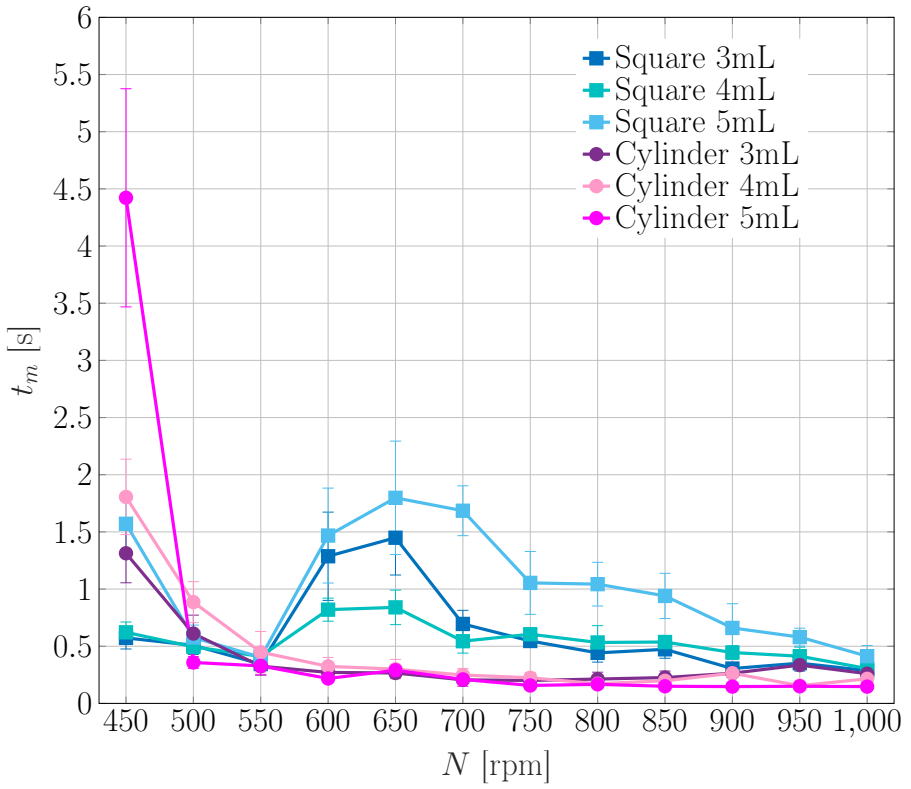
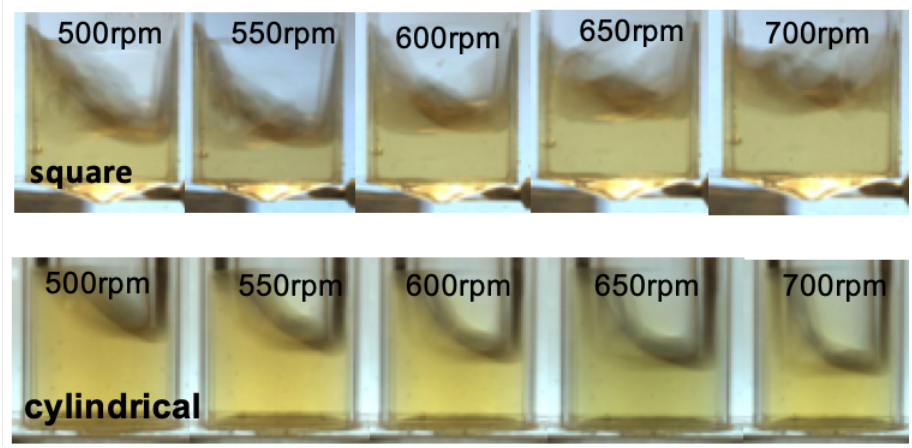


Figure 7: Comparison of mixing time variation with shaken speed between square and cylindrical geometries for three different fill volumes.



(a)



(b)

Figure 8: (a) Zoomed-in figure of the variation of mixing time with shaken speed in the range of 400 - 1000 rpm for square and cylindrical geometries. (b) Phase-locked images showing the difference of free surface shapes at certain speed ranges in the two geometries.

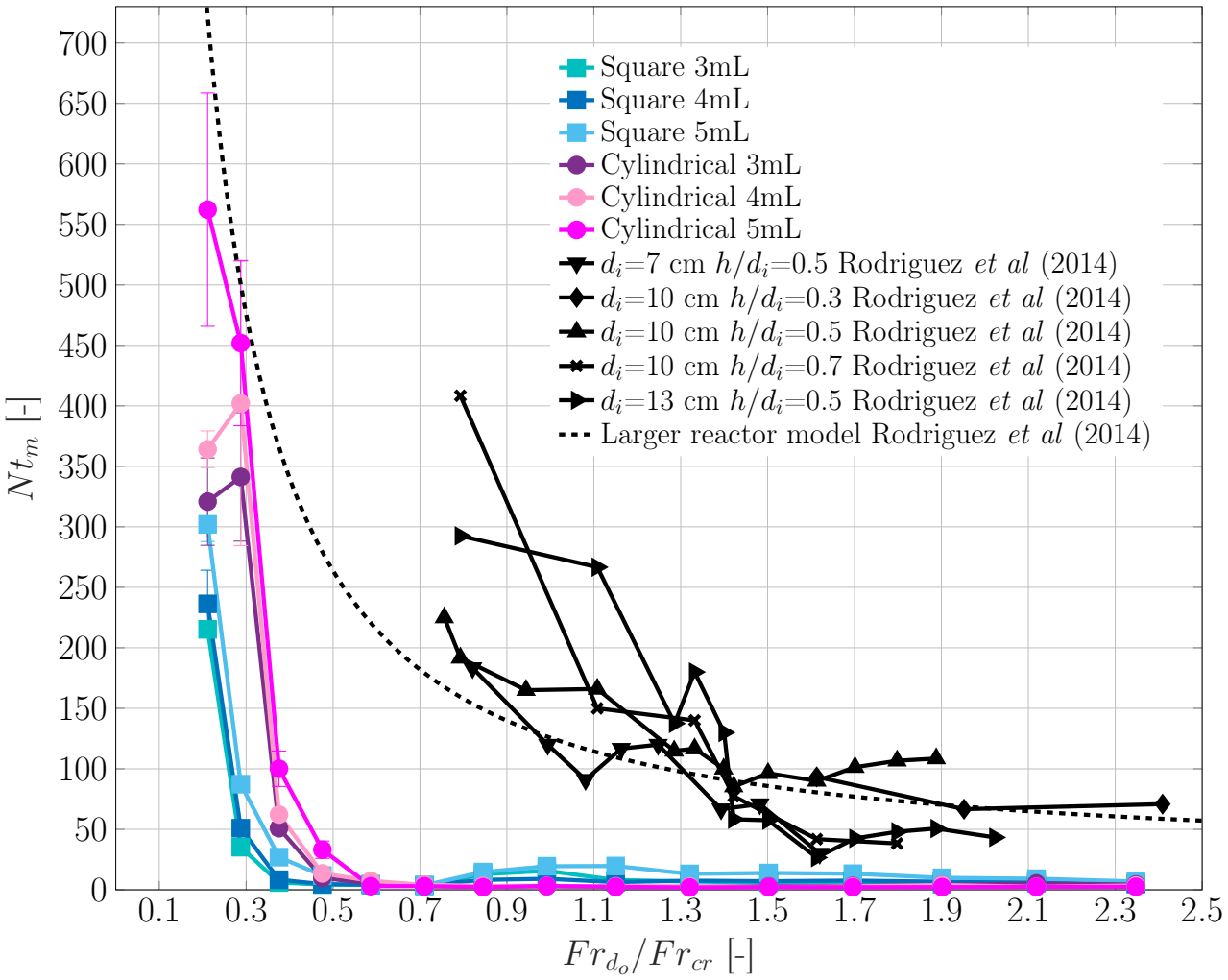


Figure 9: Comparison between present data with those reported by Rodriguez et al. [29] in terms of their proposed scaling parameter Fr/Fr_{cr}.

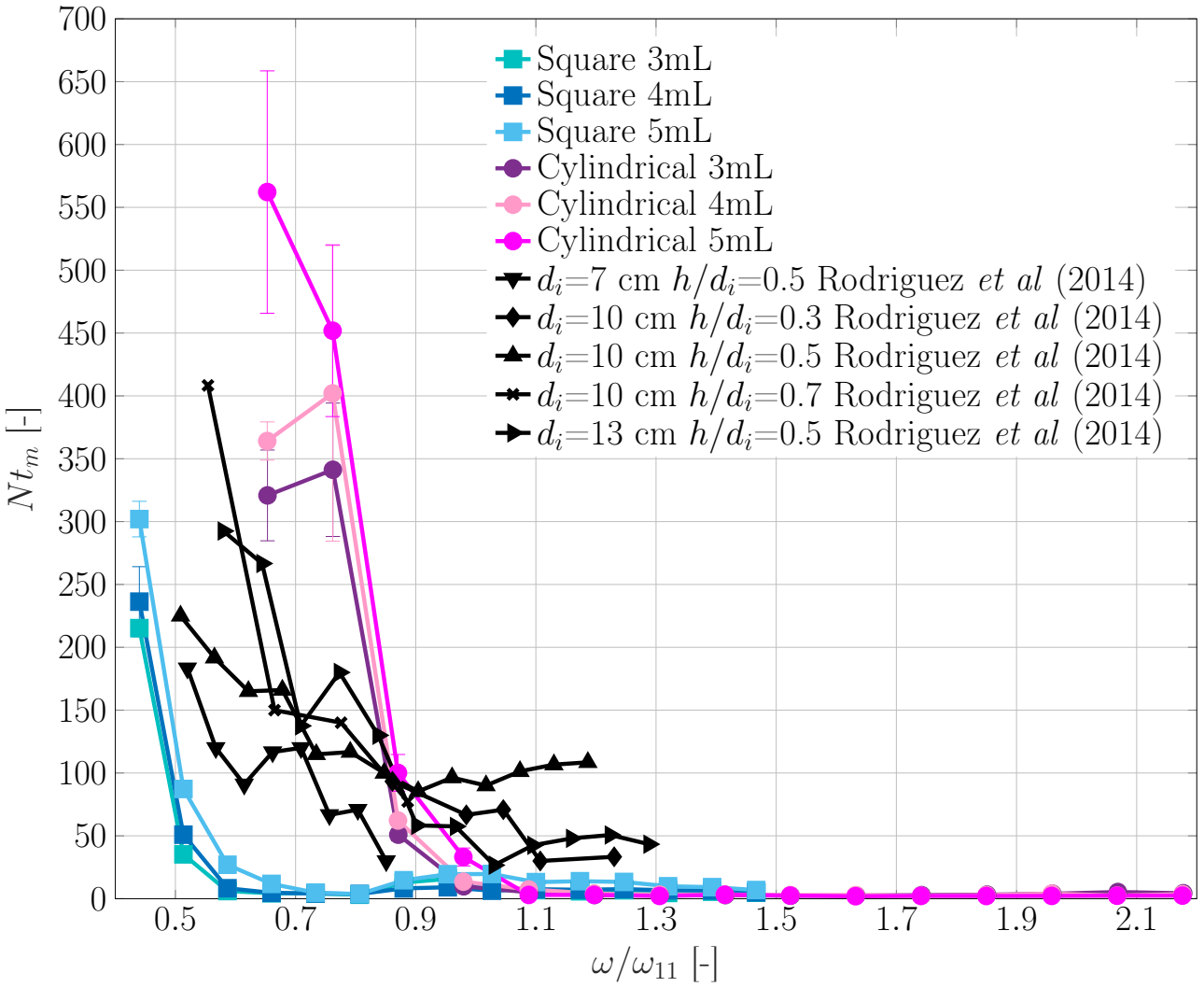


Figure 10: Comparison between present data with those reported by Rodriguez et al. [29] in terms of a new scaling parameter ω/ω_{11}.

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