

Establishment of a simple method to evaluate mixing times in a plastic bag photobioreactor using image processing based on freeware tools

Henrike Wurm

University of Applied Sciences Neubrandenburg, Brodaer Straße 2, D-17033 Neubrandenburg, Germany

Michael Sandmann (✉ sandmann@hs-nb.de)

University of Applied Sciences Neubrandenburg, Brodaer Straße 2, D-17033 Neubrandenburg, Germany

Research note

Keywords: mixing time, image analysis, photobioreactor, pH probe, aeration, algae, fluid dynamics

Posted Date: October 19th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-958895/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at BMC Research Notes on December 1st, 2021. See the published version at <https://doi.org/10.1186/s13104-021-05892-2>.

Abstract

Objective

The aim of this work was to develop a simple optical method to determine the mixing time in a photobioreactor. The image processing method should be based on freeware tools and should not require programming skills.

Results

An optical method has been established to analyze images from recorded videos of mixing experiments. The basic steps are: 1. Extraction of a sequence of images from the video file; 2. Cropping of the pictures; 3. Background removal; and 4. Image analysis and mixing time evaluation based on quantification of pixel-to-pixel heterogeneity (standard deviation over pixel intensities) within a given area of interest. The novel method was generally able to track the dependency between aeration rate and mixing time within the investigated photobioreactor. In a direct comparison, a Pearson correlation coefficient of $\rho = 0.9957$ was obtained. Gas flow rates between 10 L h^{-1} , and 300 L h^{-1} resulted from mixing times of between 48 sec and 14 sec, respectively. This simple technique is applicable even without programming skills and can be used in education within high schools and in early stages of undergraduate programs.

Introduction

Microalgae have attracted much attention due to their wide range of possible uses [1-7]. Algal cells are often grown under controlled conditions in closed photobioreactors (PBR). PBR provide better control of contamination and cell physiology than open systems, resulting in better growth and better quality of the harvested product. The major disadvantages for PBR are high initial investment costs and operating costs [8,9]. In recent years, plastic bag PBR for commercial production of microalgae have attracted considerable attention due to their low investment costs [10,11]. On the other hand, plastic bag PBR often have a reduced lifespan and inherent fragility, and they are known to suffer from inadequate mixing and decreased cell growth [12]. Proper mixing in reactors is needed, e.g., for optimal mass and heat transfer and to prevent cells settling. Inadequate mixing is the reason for the appearance of spatial gradients in reactors, which is believed to inherently increase cell-to-cell heterogeneity and thus negatively affect the productivity of bioprocesses and product quality [2,13].

Mixing time is defined as the time needed to obtain prescribed uniformity of a tracer (usually a pulse input) in a reactor that is close to a complete mixed state. In practice, a state of homogeneity with $\pm 5\%$ deviation is often the target [14]. Because of the considerable importance of this topic in industrial processes, several methods have been developed to measure mixing times, including conductivity and pH tracer experiments or colorimetric measurements [15,16]. Digital image analysis has been increasingly recognized as a valuable tool for analyzing mixing behavior in complex reactor geometries. These

techniques eliminate subjective estimates of mixing times by the human eye. One clear disadvantage is that programming skills are often needed to run the analysis automatically, thus limiting its applicability for a broader user group. In this work, a simple method based on image processing using free software tools has been established to characterize mixing behavior in a self-constructed plastic bag PBR. A “classical” pH tracer experiment was used as reference analysis.

Main Text

Experimental Section

Plastic bag photo bioreactor

Analysis was performed in a self-constructed plastic bag PBR which operates on the bubble column principle (Fig.1). Briefly, the PBR has a total height of 1.9 m and consists of a metal frame made of aluminum sections (MiniTec, Schönenberg-Kübelberg, Germany). Up to three transparent plastic bags (11 cm diameter) can be mounted on top of the frame. The bags used (CASO Design Braukmann, Arnsberg, Germany) consist of an outer polyamide layer and an inner polyethylene layer that are 15 μm and 135 μm thick, respectively. The bags are closed on the bottom with a foil heat sealer and can be filled with different volumes of water or culture media for algal growth. Six 1.16 m long dimmable light-emitting diode modules (LEDaquaristik, Hövelhof, Germany) illuminate the algal cells. The bags were aerated with 6 mm tubes made of nylon (RS Components, Frankfurt, Germany). The aeration tubes were inserted from the top of the reaction vessel and then guided to the bottom. The bags were additionally prepared with cord grips (16 mm diameter) that act as ports for sampling or integration of measuring probes, for example. The mixing time experiments were performed at room temperature (23 °C) with 10 L of distilled water and with different gas flow rates (10 L h⁻¹, 50 L h⁻¹, 100 L h⁻¹, 300 L h⁻¹). Mixing was done with nitrogen.

Reference method (pH probe)

Sodium hydroxide was used as a tracer (5 mL of 38 mM NaOH stock solution). The pH probe (EasyFerm Bio HB Arc 120 by Hamilton Bonadaz AG) was immersed 30 cm below the surface of the liquid (upper third of the liquid level) and tracked the changes in pH over time with a measurement interval of 3 sec. A manual syringe with a small tube was used to apply the solution directly to the surface of the fluid. The injection duration was a fraction of second. After the experiment was finished, the bubble column was emptied with a peristaltic pump, rinsed with distilled water, and refilled for the next experiment. Mixing time was noted as the time from addition of the tracer until $\pm 5\%$ of its final stable value (defined as the mean of the last 15 values) was reached. The reference experiments were performed in duplicate.

Optical dye tracing experiments

For these experiments, mixing of a dye was observed with a video camera and subsequently analyzed. The digital camera (Olympus OM-D E-M10 Mark II, Olympus Europa, Hamburg, Germany) was placed in front of the bubble column (mov format, 30 frames/sec). A white background was also placed behind the bubble column to minimize disturbing background signals. The blue food dye (patent blue, E131) (Brauns Heitmann, Warburg, Germany) was added with a manual syringe directly to the surface of the fluid (0.075 g powder was pre-mixed with 5 mL of distilled water). The injection duration was a fraction of second. The bubble columns were emptied after each mixing experiment with a peristaltic pump, rinsed with distilled water, and refilled for the next experiment.

Image analysis based on freeware

The key steps of the image preparation were as follows (Fig.1):

1. **Extraction** and storage of a sequence of images from the original video file (sampled at 30 images/sec) was done automatically at a frequency of one image/sec with the tool VLC media player 3.0.16 (VideoLAN, Paris, France).
2. **Cropping** of the picture was performed automatically with IrfanView 4.58 so that only the bubble columns and a reduced background area are visible.
3. Final **background removal** was performed with the on-line tool removebg (Kaleido AI, Wien, Austria). After automated processing, the files were again stored in the computer's memory and further analyzed.
4. **Image analysis** and **mixing time evaluation** rely on quantification of pixel-to-pixel heterogeneity within a given area of interest, which can be performed using the standard deviation (SD) across all pixels used. With this method, the whole image of the bubble column was used after background removal. Standard deviations were determined using Gimp 2.10.24 (GNU Image Manipulation Program). To evaluate the pixel-to-pixel heterogeneity based on the pixel intensities, the GIMP feature "value" was used. This "pseudochannel" simulates a greyscale image. Thus the color information is reduced without an additional work step.

Results

Both methods for determining the mixing times within a self-constructed 10 L bubble column PBR relied on typical tracer experiments. Exemplary data sets are shown in Figure 2. Compared to the classical pH tracer method, a novel method that is based on the use of freeware tools for image analysis was established. In both methods, the time dependent development of a signal (pH in solution or pixel-to-pixel heterogeneity within a given image) was used to determine mixing times. The measurement intervals for

the pH method and the optical analysis were 3 sec and 1 sec, respectively. Pixel-to-pixel heterogeneity, as measured by the SD, exhibits minimal values before addition of the tracer and after complete mixing. During the mixing process, SD is dynamic and relatively high.

It is seen in Figure 3 A that the mixing time decreases as the aeration rate increases but the results obtained with the optical method are always higher. The mixing times that were measured range between 48 sec and 14 sec for the optical method and between 27 sec and 12 sec for the reference method (Fig 3 A). In a direct comparison of all mixing times obtained, a relatively high linear correlation was found ($R^2 = 0.9916$).

Discussion

In this work a simple method based on image processing using free software tools has been established to evaluate mixing times in PBR. The technique was applied to a self-constructed plastic bag PBR and was compared to an established reference analysis. The optical method uses free software packages that can be applied without any programming skills and is based on characterization of pixel-to-pixel heterogeneity within a sequence of images of the mixing process within the reactor (Fig. 1). The established simple method uses the pixel-to-pixel heterogeneity, which is represented by the SD over all pixel intensities in a given area of interest. During the mixing process, the SD is very dynamic and high because the dye that is used is only partly distributed in the bubble column. As the mixed state is reached, the SD decreases and reaches a very low value after complete mixing (Fig. 2). The optical method consistently resulted in higher mixing times compared to the reference technique (Fig. 3) but was able to track the general relationship between aeration rate and mixing time as determined by the reference method. Both techniques showed a high linear correlation ($R^2 = 0.9916$). The systematically higher mixing times can be explained by two factors. First, the relatively short distance between the injection point of the tracer solution and the insertion location of pH probe. This might result in a reduced mixing time, even though complete mixing is not guaranteed. Second, the images obtained clearly show that the bottom of the bubble column is mixed in the last phase (Fig 2). Thus, the image-based technique can inherently identify segregated regions or dead zones and yields more reliable macromixing information. Another advantage of the optical method is the high time resolution, which can be as high as the intrinsic frame rate of the video. Compared to the mixing times of other PBR, the values obtained (between 48 sec and 14 sec for the optical method) are relatively low, indicating good mixing behavior. In a rectangular airlift loop PBR, mixing times of 30 to 200 sec have been described [17]. Even higher mixing times of 5 minutes are possible in tubular and flat panel PBR [18]. In relation to other image-based methods [16,19,29], the described method can be implemented without commercial software and programming skills. Additionally, no advanced mathematical data handling steps are needed, such as discrimination between mixed and unmixed pixel populations [19]. Despite the simplicity of the image-based method, it can be applied only in transparent reactors like PBR.

Limitations

No replicate experiments were performed for the evaluation of the mixing times with the optical method. Nevertheless, the data acquired seem to be strongly comparable to the reference analysis.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The original datasets and other miscellaneous materials related to the investigation are available upon reasonable request to the corresponding author.

Competing interests

The authors declare that they have no conflict of interests.

Funding

We acknowledge the financial support of the University of Applied Sciences Neubrandenburg (internal research funding grant 13311002)

Author's contributions

HW and MS conducted the experiments, collected data, and analyzed the dataset. MS contributed with supervision and gathering of the funds. MS and HW prepared the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We like to acknowledge Siegfried Bolenz for assistance in the initial planning phase of the experiments.

Abbreviations

sec: Second

PBR: Photobioreactor

SD: Standard deviation

Fig: Figure

References

1. de Jaeger L, Verbeek RE, Draaisma RB, Martens DE, Springer J, Eggink G, Wijffels RH. Superior triacylglycerol (TAG) accumulation in starchless mutants of *Scenedesmus obliquus*: (I) mutant generation and characterization. *Biotechnol. Biofuels*. 2014;7:69.
2. Sandmann M, Schafberg M, Lippold M, Rohn S, Analysis of population structures of the microalga *Acutodesmus obliquus* during lipid production using multi-dimensional single-cell analysis. *Sci. Rep*. 2018;8:6242.
3. Wells ML, Potin P, Craigie JS, Raven JA, Merchant SS, Helliwell KE, Smith AG, Camire, ME, Brawley SH. Algae as nutritional and functional food sources: revisiting our understanding. *J. Appl. Phycol*. 2017;29:949–982.
4. Zhu L, Biorefinery as a promising approach to promote microalgae industry: An innovative framework. *Renew. Sust. Energ. Rev*. 2015;41:1376–1384.
5. Smetana S, Sandmann M, Rohn S, Pleissner D, Heinz V. Autotrophic and heterotrophic microalgae and cyanobacteria cultivation for food and feed: Life Cycle Assessment. *Bioresour. Technol*. 2017;245:162–170.
6. Sevgili H, Sezen S, Yılayaz A, Aktaş Ö, Pak F, Aasen I M, Reitan K I, Sandmann M, Rohn, S, Turan G, Kanyılmaz M. Apparent nutrient and fatty acid digestibilities of microbial raw materials for rainbow

- trout (*Oncorhynchus mykiss*) with comparison to conventional ingredients. *Algal Res.* 2019;42:101592.
7. Hensel B, Jakop U, Scheinplflug K, Schröter F, Sandmann M, Mühldorfer K, Schulze M. Low temperature preservation: influence of putative bioactive microalgae and hop extracts on sperm quality and bacterial load in porcine semen. *Sustain. Chem. Pharm.* 2021;19:100359.
 8. Gupta PL, Lee SM, Choi HJ. A mini review: photobioreactors for large scale algal cultivation. *World J. Microbiol. Biotechnol.* 2015;31:1409–1417.
 9. Gifuni I, Pollio A, Safi C, Marzocchella A, Olivieri G. Current bottlenecks and challenges of the microalgal biorefinery. *Trends Biotechnol.* 2018;37:242–252.
 10. Schreiber C, Behrendt D, Huber G, Pfaff C, Widzowski J, Ackermann B, Müller A, Zachleder V, Moudříková Š, Mojzeš P, Schurr U, Grobbelaar J, Nedbal L. Growth of algal biomass in laboratory and in large-scale algal photobioreactors in the temperate climate of western Germany. *Bioresour. Technol.* 2017;234:140–149.
 11. Wang B, Lan CQ, Horsman M. Closed photobioreactors for production of microalgal biomasses. *Biotechnol Adv* 2012;30:904–12.
 12. Huang Q, Jiang F, Wang L, Yang C. Design of photobioreactors for mass cultivation of photosynthetic organisms. *Engineering* 2017;3:318–329.
 13. Enfors SO, Jahic M, Rozkov A, Xu B, Hecker M, Jürgen B, Krüger E, Schweder T, Hamer G, O’Beirne D, Noisommit-Rizzi N, Reuss M, Boone L, Hewitt C, McFarlane C, Nienow A, Kovacs T, Trägårdh C, Fuchs L, Revstedt J, Friberg PC, Hjertager B, Blomsten G, Skogman H, Hjort S., Hoeks F, Lin HY, Neubauer P, van der Lans R, Luyben K, Vrabel P, Manelius Å. Physiological responses to mixing in large scale bioreactors, *J. Biotechnol.* 2001;85:175–185.
 14. Nienow AW. On impeller circulation and mixing effectiveness in the turbulent flow regime. *Chem. Eng. Sci.* 1997;52:2557–2565.
 15. Rosseburg A, Fitschen J, Wutz J, Wucherpfennig T, Schlüter M. Hydrodynamic inhomogeneities in large scale stirred tanks—Influence on mixing time. *Chem. Eng. Sci.* 2018;188:208–220.
 16. Cabaret F, Bonnot S, Fradette L, Tanguy PA. Mixing Time Analysis Using Colorimetric Methods and Image Processing. *Ind. Eng. Chem. Res.* 2007;46:5032–5042.
 17. Guo X, Yao L, Huang Q. Aeration and mass transfer optimization in a rectangular airlift loop photobioreactor for the production of microalgae. *Bioresour Technol.* 2015;190:189–95.
 18. Oncel S, Kose A. Comparison of tubular and panel type photobioreactors for biohydrogen production utilizing *Chlamydomonas reinhardtii* considering mixing time and light intensity. *Bioresour. Technol.* 2014;151:265–270.
 19. Vega-Alvarado L, Taboada B, Hidalgo-Millán A, Ascanio G. An Image Analysis Method for the Measurement of Mixing Times in Stirred Vessels. *Chem. Eng. Technol.* 2011;34:859–866.
 20. Melton L, Lipp C, Spradling R, Paulson KA. “Dismt - Determination of mixing time through color changes.” *Chemical Engineering Communications* 2002;189:322–338.

Figures

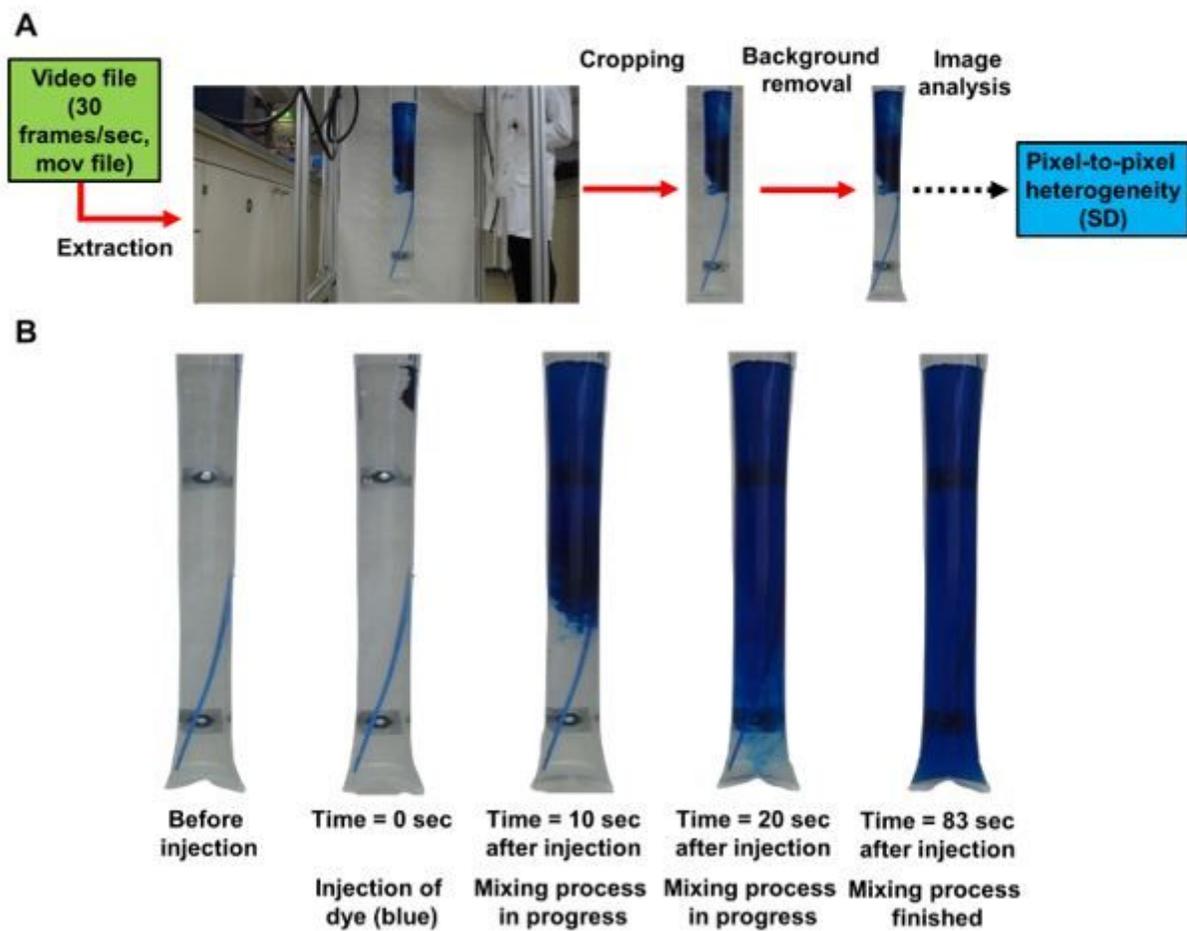


Figure 1

Experimental details. A Image processing flow chart. B Example of frames extracted from a sequence of images captured during a mixing process (gas flow rate 10 L h⁻¹)

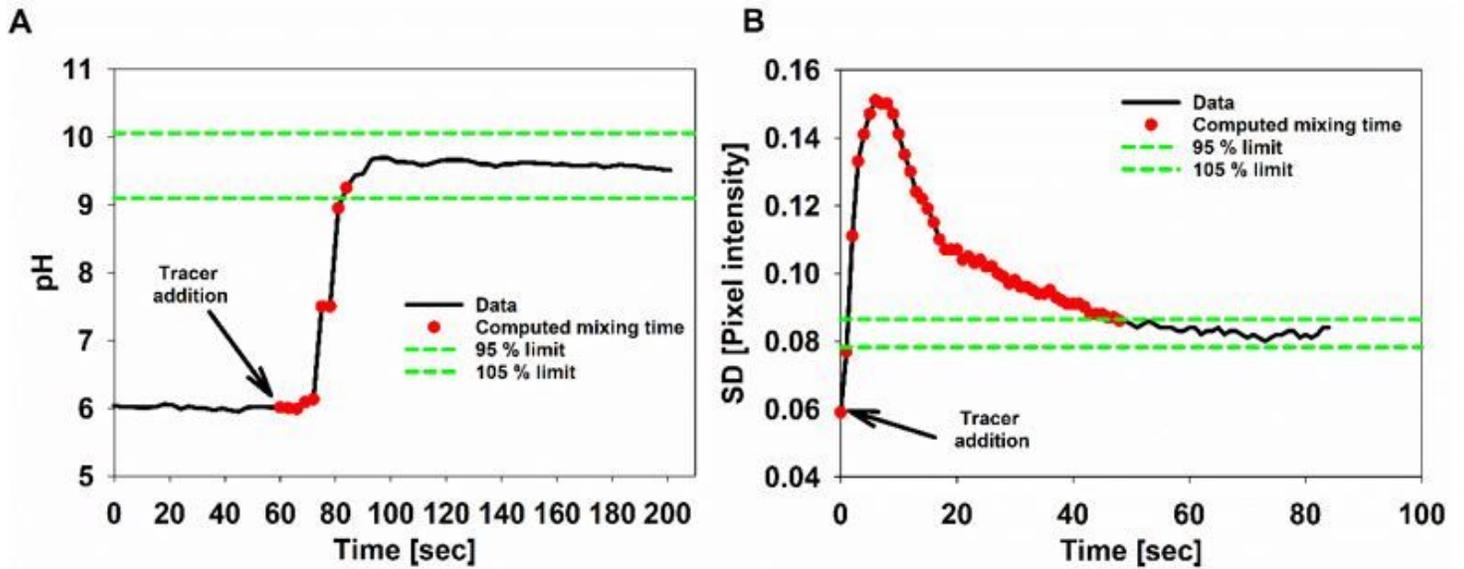


Figure 2

Determination of mixing times in the 10 L bubble column at a gas flow rate of 10 L h⁻¹. The mixing times have been evaluated between tracer additions until signal stability of $\pm 5.0\%$ was reached (evaluated length of mixing time marked with red dots). A Reference method (pH probe). B Optical dye tracing method.

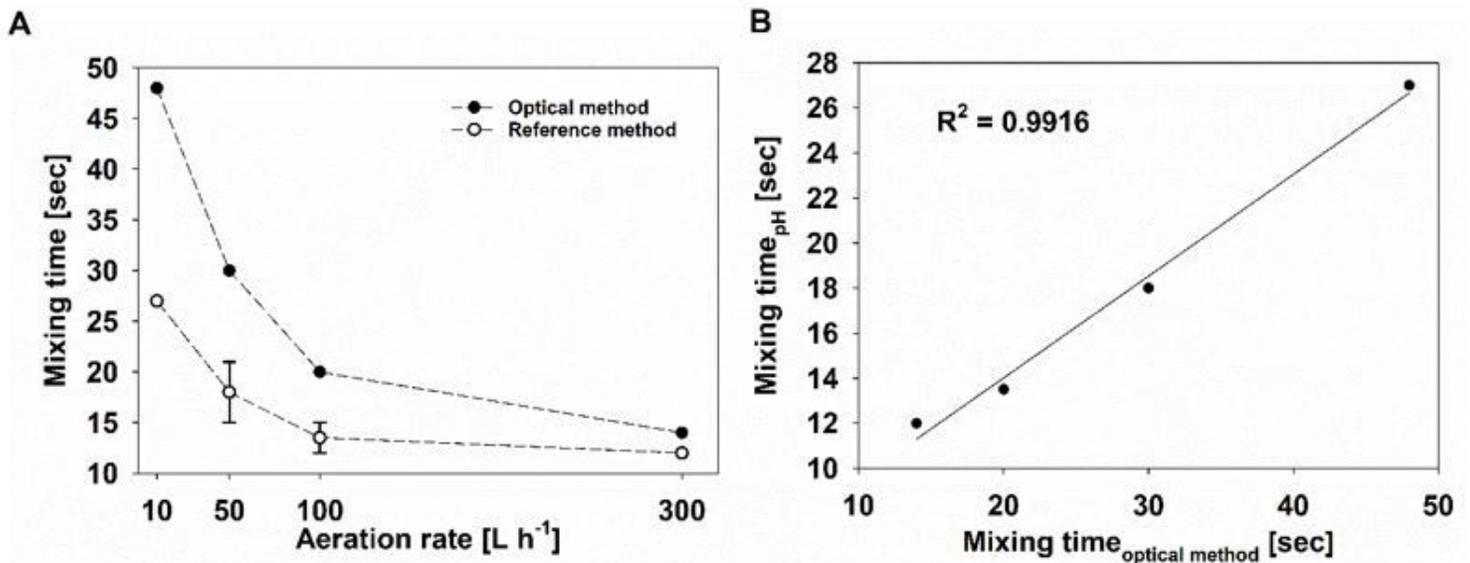


Figure 3

Comparison of methods. A Effect of different aeration rates on mixing time (mean and standard deviation are shown for reference analysis, n = 2). B Correlation of mixing times derived from optical dye tracing method with reference analysis.