

Application data sheet #03

Constant temperature incubator shaker **Bio-Shaker BR Series**

Relationship between the shaking speed and the amplitude, as seen from volumetric oxygen transfer coefficient ($k_L a$)



Re-examining the established theories and practices for shaking culture with cutting-edge technology (Introduction 2)

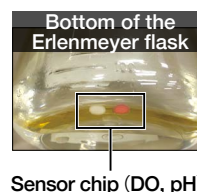
Overview

With the aerobic culture of microorganisms, an oxygen supply to culture solutions frequently becomes a bottleneck of culture efficiency. It is often evaluated with the volumetric oxygen transfer coefficient in the liquid phase ($k_L a$).^{*} $k_L a$ is a parameter of the speed for oxygen under the gas phase to melt into the liquid phase, or a culture solution, i.e. the quality of aeration. However, it is known that in a normal shaking culture without active gas ventilation, when carbon dioxide generated by microorganisms' aerobic breathing is not discharged out of the container, air exchange is interrupted, thereby resulting in an anaerobic state (see ADS #02). Therefore, though culture efficiency is not determined solely by $k_L a$ in a normal shaking culture, it can be considered as an indicator for reference. As many constant temperature shaking incubators can change amplitudes, we often receive questions about the relationship between the shaking speed and the amplitude, in addition to the relationship between amplitude and culture efficiency. One may find information on $k_L a$ changes depending on the differences in container size, liquid volumes, and shaking speeds on the Internet, but we did not find any investigative results including difference in amplitude. This sheet shows the results of actually investigating the impact of amplitude on $k_L a$ by using SFR, DO, and pH measurement system for Erlenmeyer flasks.

^{*} For gas transfer from gas to the liquid phase, the Lewis and Whitman Two-film Theory is used¹⁾. In other words, a two film gas-liquid interface as a border, one film on the gas phase and one film on the liquid phase, and the transfer speed from the gas phase to the liquid phase (overall volumetric oxygen transfer coefficient: $k_L a$) depends on the transfer speeds within the respective boundary films. Normally, the gas transfer on the gas phase side is extremely large compared to the liquid phase, not causing any resistance. It is measured and used as the transfer speed on the liquid phase (liquid-phase volumetric oxygen transfer coefficient: $k_L a$).

About SFR

SFR is a piece of equipment that can implement non-contact monitoring of dissolved oxygen (DO) and Hydrogen Ion Concentration Index (pH) in conjunction with the time of fluorescence loss using multiple Erlenmeyer flasks at the same time. It can be easily used by mounting it on a BioShaker, etc. Data is transmitted to a PC wirelessly. Measurements require a consumable Sensor Flask ("Erlenmeyer flask") but are useful as individual parameters in shaking culture become clear. In this experiment, we used SFR and a BioShaker to make a calculation ($k_L a$ values when Erlenmeyer flasks in different sizes are shaken with a different set of shaking speeds and amplitudes).



G-BR-300 BioShaker fixed on the shaking rack. ↑
Erlenmeyer flasks (Sensor Flasks) of 125 mL to 2 L can be used by replacing clamps, and a maximum of nine Erlenmeyer flasks can be mounted (depending on sizes). For further details, please visit our website.

Measurement and calculation method of $k_L a$ with sodium sulfite method and its results

Temperature, container, and sample +37 °C ,

Erlenmeyer flask, anoxic water whose volume is equivalent to 33% of container (v/v)

Two grams of sodium sulfite were dissolved into tap water to make one liter of anoxic water, which was then put into individual Erlenmeyer flasks. The flasks were mounted on the installed SFR to the BioShaker in order to monitor the changes of dissolved oxygen concentration. From the obtained data, the following calculation formulas^{1, 2)} were used to calculate $k_L a$.

$k_L a$ [1/hr] when the saturated dissolved oxygen concentration is set to 100% and the concentration is changed from 10% to 90%

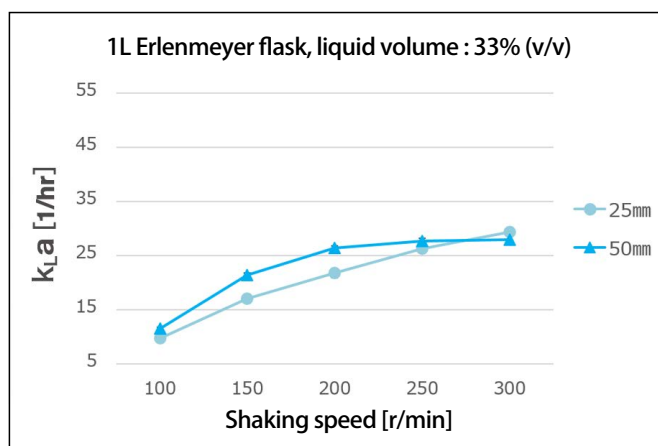
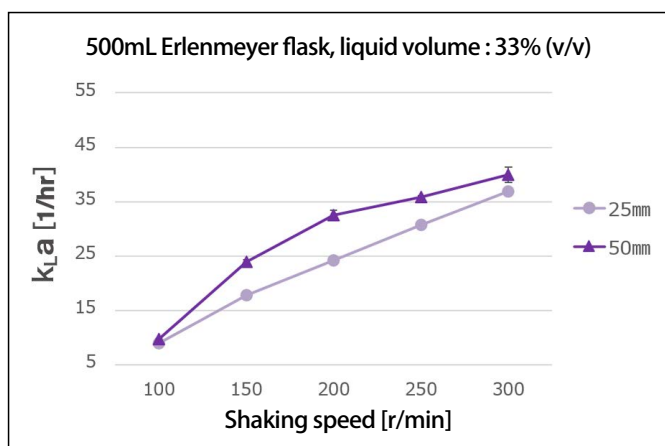
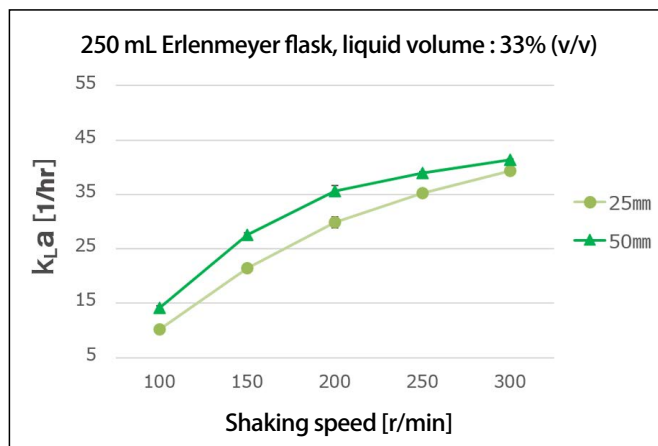
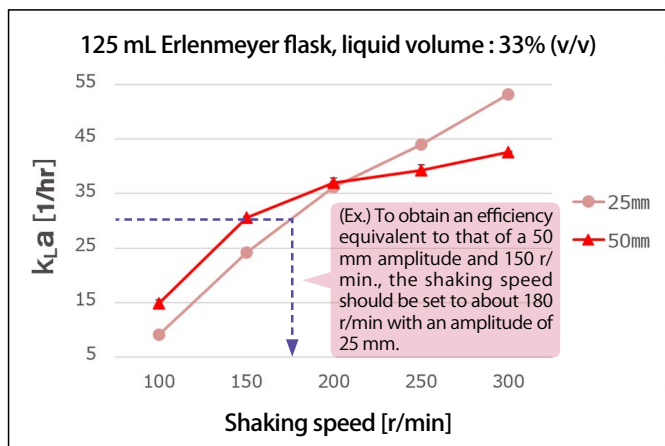
$$k_L a = \ln \{ (100 - 10) \div (100 - 90) \} \div (t_2 - t_1) \times 60$$

\ln is a natural logarithm. t_1 is the time required to reach 10% (min.), while t_2 is the time required for 90% (min.) However, affected by intervals (10 seconds in this experiment), both the concentration and the time vary somewhat, resulting in slight variations across individual experiments. The table on the right shows sets of $k_L a$ based on shaking conditions, and each value is an average of the results of four or more experiments. (For easy viewing, values are rounded off to whole numbers.) Graphs by the size of an Erlenmeyer flask can be found on the back of this sheet.

	Erlenmeyer flask	Shaking speed	Amplitude	Amplitude
			(25 mm)	(50 mm)
$k_L a$ [1/hr]	125mL	100r/min	9	15
		150r/min	24	31
		200r/min	36	37
		250r/min	44	39
		300r/min	53	43
250mL	250mL	100r/min	10	14
		150r/min	21	28
		200r/min	30	36
		250r/min	35	39
500mL	500mL	300r/min	39	41
		100r/min	9	10
		150r/min	18	24
		200r/min	24	33
1L	1L	250r/min	31	36
		300r/min	37	40
		100r/min	10	12
		150r/min	17	21
		200r/min	22	26
		250r/min	26	28
		300r/min	29	28

● The graphs take advantage of the relationship between the shaking speed and the amplitude in the shaking culture

Upon preparing a graph of $k_L a$ obtained for each size of Erlenmeyer flasks, it was found that we could avoid compensating for a small amplitude with a shaking speed or meaninglessly increasing the shaking speed. Basically, the smaller the size of the container the faster the shaking speed, and the bigger the amplitude the larger the $k_L a$, but for 125 mL flasks, the relationship between 25 mm and 50 mm was reversed with a border of 200 r/min. A similar reversal phenomenon was likely to appear for a 1L flask with a border of 300 r/min. As we could not prepare an incubator shaker capable of switching amplitudes that could shake the SFR and 1L load at 301 r/min. or higher, and with an amplitude of 50 mm; we could not confirm this phenomenon. (If we use our BR-180LF, tests at 400 r/min. it is possible with the limitation of 25 mm of amplitude and rotary shaking only.) To utilize this result, please keep in mind the possibility of this reversal phenomenon and refer to the graph as necessary.



Discussion

The individual reliability of a few $k_L a$ measurement methods that are available is omitted here, but what was revealed by this experiment was that $k_L a$ can easily change due to a factor other than target parameters such as shaking speeds and amplitudes. To be more precise, the manner in which the Erlenmeyer flask is fixed. When the experiment started, we tried to shake the flask without using the spring of the clamp at a low speed. We found that the reproducibility of $k_L a$ was low. In some cases, the value obtained from a larger Erlenmeyer flask was bigger than that obtained from a smaller flask. After noticing this, we were able to improve the reproducibility. For this experiment, as the purpose of observing the relationship between shaking speeds and amplitudes using $k_L a$ as an indicator, the reproducibility of $k_L a$ was important, but as it can easily vary with such a minor cause, when using this value for reference in an actual shaking culture or when calculating the value on one's own, it would appear to be better to consider it as a relative value dependent on container sizes and other parameters such as liquid volume, shaking speed, and amplitude, rather than an absolute value. We therefore add that, if any additional test is conducted with a different incubator shaker or methods to fix the container, the result values may be different from the ones shown above. (Values may also change depending on measurement methods'.)

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References

- 1) Journal of Environmental Conservation Engineering, Vol. 11 No. 10 (1982), 739-745 (Society of Environmental Conservation Engineering)
- 2) Imanaka, Tadayuki et al, Great Development of Microorganisms, 1st Ed. 2002, 544-545 (NTS)

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For inquiries about the BioShaker and the contents of this leaflet, please contact us as provided on the left.