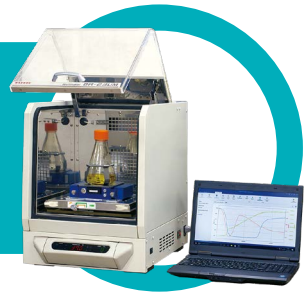
Application data sheet #02

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

About the method for shaking Erlenmeyer flask and aeration efficiency



Re-examining the established theories and practices for shaking culture with cutting-edge technology

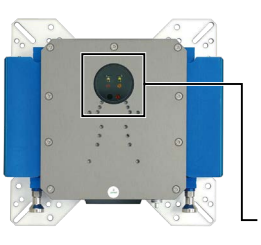
Overview

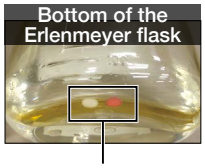
When it comes to shaking culture methods of microorganisms, there are two common methods. A method to shake an Erlenmeyer flask with a rotary shaker, which was developed in the Netherlands in 1933, while another method to shake a shake (Sakaguchi) flask with a reciprocal shaker was devised in Japan in 1942 1). Because of this background, rotary shakers are mostly used overseas, while in Japan, in addition to such shakers, reciprocal shakers are used when shaking culture is conducted in a test tube or when high culture efficiency is desired in a shake flask. (It is very rarely that shake flasks are used overseas).

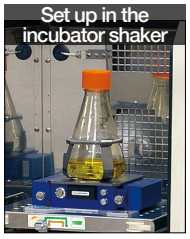
When reading textbooks on culturing microorganisms, it is said that an Erlenmeyer flask is most suitable for rotary shaking and a shake flask is most suitable for reciprocal shaking, respectively. At TAITEC, we are often asked by customers, "When shaking with an Erlenmeyer flask, which one offers higher aeration efficiency, rotary or reciprocal?" Because established theories in textbooks appear to be too out-of-date, we could not find any quantitative proof that can be quoted to answer this question. Therefore, we decided to actually examine this by using an SFR Vario.

About SFR Vario and method for quantitative check

An SFR Vario is a piece of equipment that can implement non-contact and real-time monitoring of not only dissolved oxygen (DO) and Hydrogen Ion Concentration Index (pH), but also the optical density (OD) in conjunction with the time of fluorescence loss. It can be easily used by mounting it on a TAITEC BioShaker, etc. Data is transmitted to a PC wirelessly. The disadvantages of this method includes the consumables required for DO and pH measurements and a value of less than one when converted to OD600 (specification in which users should draw a calibration curve and input an approximate value calculated) is not within the range of guaranteed measurements as the OD measurement is based on reflective light methods. This method is still worth paying attention to because the individual parameters of shaking culture that have been difficult to quantify can be made clear. In this case, by monitoring DO, we were able to quantify the difference across the aeration efficiency between rotary shaking and the reciprocal shaking of an Erlenmeyer flask by using an SFR Vario and a BioShaker.

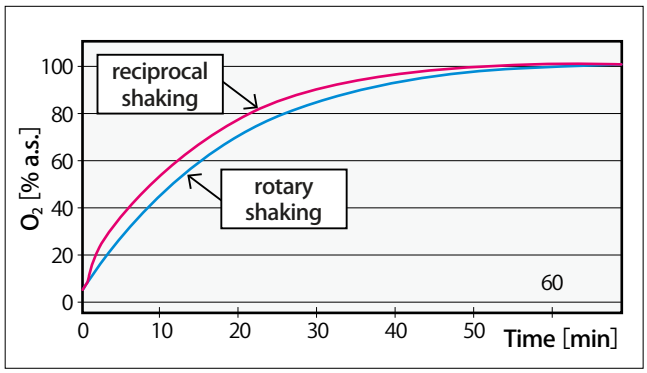






Results and discussion

Constant temperature incubator shaker:
BioShaker BR-23FP (reciprocal and rotary shaking are switchable)
Shaking speed, amplitude, and temperature:
150 r/min., 25 mm, +37° C
Sample: anoxic water, 150 mL, in a 500-mL Erlenmeyer flask

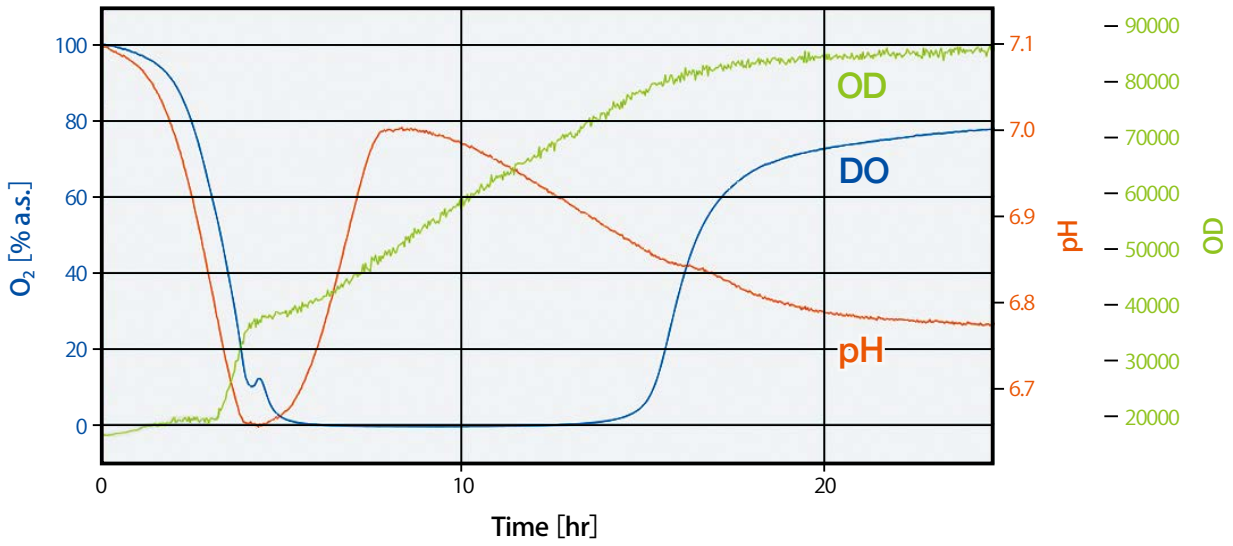


Tap water that is in an almost anoxic state by being aerated to nitrogen gas is put into an Erlenmeyer flask. We then placed the flask as well as an SFR Vario in a BioShaker and monitored the changes of the DO density by shaking. The results showed that aeration is slightly better with reciprocal shaking. When the Erlenmeyer flask was shaken in a reciprocal manner, in some cases the behavior of the liquid was similar to that of rotary shaking, and in other cases the liquid splashed harshly on the attached stopper of the Erlenmeyer flask. This was because of the amount of liquid and the speed of shaking against the volume of the Erlenmeyer flask. For the shaking culture with rotary shaking of an Erlenmeyer flask, it turned out that the speed of the increase in optical density is accelerated in proportion to the speed of shaking and the inverse proportion to the amount of liquid (see ADS #25). Reciprocal shaking yielded unevenness and disadvantages based on conditions. With preference to constant speed rather slightly higher speed in terms of dissolution speed, the established theory says that rotary shaking is more suitable for an Erlenmeyer flask.

What happens when the optical density is compared and examined? We could not conduct this experiment with conditions equal to this examination. We will introduce interesting data on the next page. (See back side of this sheet.)

Reference

Container and culture medium: 500 mL Erlenmeyer flask (disposable type with a DO/pH sensor chip), YPD 100 mL
Shaking speed, amplitude, and temperature: 120 r/min., 25 mm, +30° C



The above are the results of monitoring DO, pH and optical density (OD) in the shaking culture of yeast (Saccharomyces cerevisiae) obtained by the SFR Vario. The reference to pH is omitted herein, with a focus on DO and optical density. After starting the culture, DO quickly decreased with an increasing optical density, but never increased until the optical density reached a plateau. This suggests that the supply of DO could not keep up with the increasing yeast. Shaking culture tends to be considered as an aerobic culture condition, yet we can clearly see that it is close to an anaerobic condition. This is likely to be well-known in the field of fermentation control, but in shaking culture methods that are frequently used for applications where no large amounts of bacterial cells are required such as gene cloning, in addition to classification and identification in molecular biology and microbiology, not much attention has been paid to this. It is known that while yeast easily grows in an aerobic condition, it shifts its metabolism to ethanol generation from growth in an anaerobic condition. Dr. Tanaka et al.2), pointed out that the main reason DO supply is insufficient in shaking is because in addition to the low solubility of oxygen, carbon dioxide exhausted by the subject of a culture (yeast herein) as a result of metabolism, which disturbed the dissolution of oxygen. In other words, poor ventilation efficiency of air within the container. Based on this specific knowledge, we plan to explore methods for improving this ventilation efficiency within the container.

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References

1) Tanaka, Hideo, *Journal of Bioscience and Bioengineering*, 84, 2-15 (2006)
2) Tanaka, Hideo and Yoko Ogawa, Patent Application #141796 (2001)

Publication: 2017

For inquiries about the BioShaker BR-23UM and the contents of this leaflet, please contact us as provided on the left.