Analysis and application of the influence of accumulating CO_2 in the headspace during shake-flask culture on microorganism and microbial community

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Report:

Accumulation of CO_2 in the gas phase in microbial culture was shown to affect the shake-flask culture. CO_2 accumulation was found to be dependent not only on the shape of the flask, but also on the type of breathable culture-stopper. We also demonstrated that the new culture method in which the CO_2 concentration in the flask gas phase is controlled constantly or intermittently, presented different microbial behavior from that of the conventional method, illustrating that this method may affect various physiological activities.

Research aims

It was difficult to investigate and control culture factors in a shake-flask culture without the interruption of shaking, because of high speed of revolutions [1)]. Therefore, it is difficult to elucidate the effects of various flask conditions (e.g.., type of culture-stopper, flask shape, and shaking conditions), and consequently, these conditions have not been studied. In order to maximize the potential of shake-flask culture, some techniques are necessary for monitoring culture environments in flasks and selecting appropriate flask conditions. This study focused on CO_2 produced during microbial culture, using a developed Circulation Direct Monitoring and Sampling System (CDMSS); wherein sampling and monitoring are possible without interrupting shaking [2)] (and references therein). CDMSS was used to evaluate the ventilation capacity of the entire incubator comprising of a conventional flask with a culture-stopper (quantification of the total gas transfer rate between the flask gas phase and the outside air) and creation of a novel flask condition. In addition, this study also analyzed the effects of novel flask conditions on microorganisms.

Methods

After filling the flask gas phase with CO_2, CO_2 concentration was monitored using CDMSS using culture-stoppers with varying air permeability in combination with various flasks, and the time at which the CO_2 concentration in the flask was halved was calculated. In addition, we developed a unique system which can control the CO_2 concentration in the flask gas

phase by connecting the CDMSS and aeration device with a PID control device using the thus obtained CO_2 ventilation capacity. This system was able to function using a conventional culture-stopper and flask. This newly developed CO_2-controlled method for shake-flask culture was compared with the conventional method.

Results

The ventilation capacity and oxygen supply capacity of Erlenmeyer flasks, Sakaguchi flasks, and cylindrical flasks were evaluated for the purpose of creating novel flask conditions [3]. Results of our study on shaking cultures of *Escherichia coli* using various flask shapes showed that not only the oxygen supply capacity, but also the ventilation capacity is important (**Fig. 1**) [3]. The half-life of CO_2 was used as an index to evaluate the ventilation performance of plug-type and cap-type culture-stoppers made of silicone foam, which are widely used as breathable culture-stoppers. The CO_2 half-life values obtained for both stoppers were different (**Fig. 2**), thus it was clear that this difference affects the growth of microorganisms [4]. A significant difference was observed in the culture-stopper depending on the material. There was almost no change in the half-life of CO_2 due to shaking conditions. In order to compare the newly developed CO_2-controlled shake flask culture method with the conventional method, a pure culture of *Pelomonas saccharophila* showed increased growth upon controlling the high concentration of CO_2 in the flask gas phase [4]. As a factor that increases the CO_2 concentration in the flask gas phase, we studied flame sterilization operation at the time of sampling in the next experiment. We showed that high concentrations of CO_2 accumulate in the flask gas phase by utilizing a measurement imitating the actual operation of flame sterilization and simulation of combustion gas by

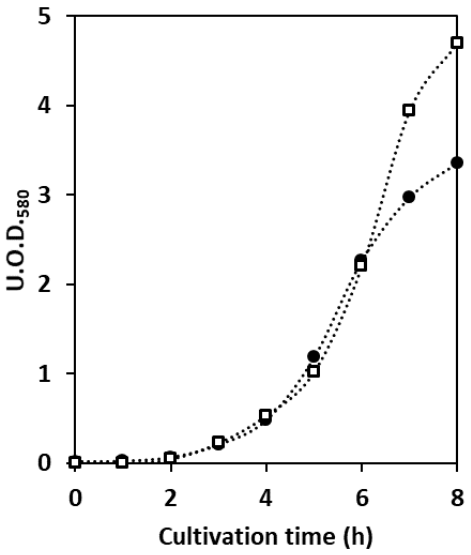

Fig. 1 Growth course of *E. coli* in shake culture with equivalent oxygen supply capacity but different ventilation capacity. ●, Erlenmeyer flask; □, Cylindrical flask.

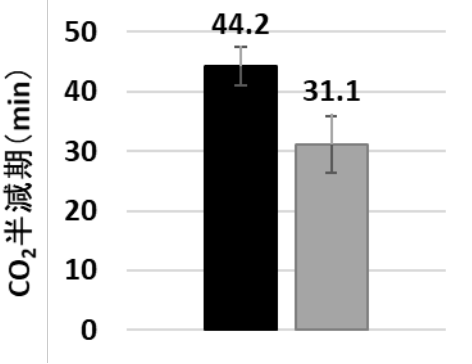

Fig. 2 Ventilation capacity of the breathable culture-stopper. Black, plug type; ash, cap type.

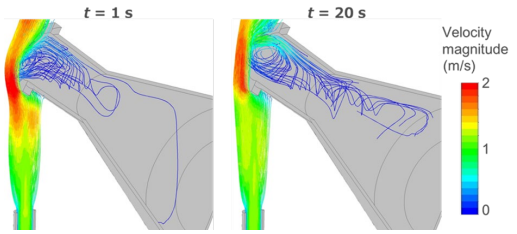

Fig. 3 Behavior of combustion gas containing high concentration CO_2 generated by flame sterilization operation in the sampling process.

CFD (**Fig. 3**). Shaking culture was performed by mimicking only the intermittent increase in CO_2 concentration seen during sampling over time, and as a result, *Acetobacter pasteurianus*, *P. saccharophila*, cultures showed increased growth, however, *E. coli* and *Saccharomyces cerevisiae* cultures did not show this change [5].

Conclusion

It is necessary to select breathable culture-stoppers based on to microbial culture conditions including the medium used. In the selection of culture-stoppers, it was shown that their shape (such as cap-type and plug-type) and material, and the foaming method used, allow reproducible microbial culture. The flask environment of the gas phase during aerobic conventional shake culture is an important culture factor along with the culture medium used. The constant or intermittent control of the CO_2 concentration in the headspace provides novel flask conditions. It is suggested that shake-flask culture allowing CO_2 accumulation affects the growth and various physiological activities, and it is expected to contribute to upstream bioprocess development wherein the shake-flask culture method is widely used. In addition, as a model of the microbial flora, moromi was cultured under various gas phase conditions. As a result, the culture microbial community structure obtained was different from the conventional one, depending on the conditions of flask gas phase.

References

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