(Short Communication)

Effects of Liquid Film Formed on Flask Surface on Oxygen Transfer Rate in Shaking Flask and Development of Baffled Shaking Vessel by Optical Method Based on Sulfite Oxidation

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ABSTRACT

The gas liquid mass transfer volumetric coefficients K_La in several kinds of small size shaking flasks were measured with an optical method based on sulfite oxidation without oxygen concentration probe and liquid sampling. It was found that the liquid film on the inner surface of flask was one of very important factors to increase K_La. The baffled flask was effective to obtain larger Oxygen Transfer Rate than normal flask. A new developed baffled cylindrical vessel based on the baffled small flask has about three times larger K_La than the normal vessel.

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Introduction

Shake mixing has been widely used in plant and animal cell culture. A shaking flask has many advantages, that is, it is easy to handle, it can be operated as a closed system, and the cell is not damaged because the flask has not an impeller, which is used in a standard stirred tank reactor. Thus contamination is reduced and good fermentation results are obtained. For cell cultures by shaking flasks, it is much important to estimate both the oxygen transfer rate from the liquid surface to the bulk liquid and from the bulk liquid to the cell surface. Maier (2002) measured the gas liquid mass transfer volumetric coefficient K_La in Erlenmeyer shaking flasks and correlated K_La with several operation parameters (shaking diameter, shaking speed, flask size and filling volume). Kato *et al.,* (1997) measured K_La in large scale cylindrical shaking vessels and correlated K_La with the power consumption per unit volume, the vessel size and the liquid height. However, because both the correlations were obtained empirically, they should not be used outside of the experimental conditions.

There are many types of shaking flasks, for example, Erlenmeyer, spherical and cylindrical vessels. In this work, the gas-liquid mass transfer volumetric coefficient K_La of some kinds of Erlenmeyer and cylindrical flasks were observed. The effects of the liquid film on the inner surface of flask on K_La were examined. In addition, the effect of baffled flask on K_La was examined to develop a new baffled cylindrical shaking vessel which has larger K_La than a normal cylindrical shaking vessel.

1. Experimental

Erlenmeyer and cylindrical flasks made of glass were used with a commercial shaker (NR-80, TAITEC Co. Ltd.). The maximum inner diameter D and the top diameter d_i were 95－115 mm and 16－40 mm, respectively, as shown in **Figure 1**. The liquid volume V_L in the flask, the shaking diameter d and the shaking speed N were 50－200 m*l*, 10－40 mm and 120－200 rpm, respectively. The wettability on the flask surface was controlled with coating materials which prevented the forming water film on the inner flask surface. Baffled flasks and a new baffled larger vessels which were developed in this study were shown in Figure 1.

The mixing fluid used was a sodium sulfite solution as described below.

1.1 Optical method to measure gas liquid mass transfer volumetric coefficient

There are two ordinary methods to measure the gas-liquid mass transfer volumetric coefficient K_La; i.e., dissolved oxygen concentration method and titration method. However, both the methods cannot be used for small size flasks like those in the present study, because the probe of the dissolved oxygen meter cannot be put in the flask and because the liquid sampling affects the liquid volume in the flask. Thus an alternative method must be used to measure K_La for small size flasks.

An optical method to measure the oxygen transfer rate *OTR* in a small size flask was established by Hermann *et al* .,(2001). It was based on sulfite oxidation which made a shift in pH when the total amount of sulfite was converted to sulfate. The shift in pH was indicated by a pH sensitive dye – bromthymol blue. The time from the start of the experiment to the color shift t_{ox} was measured, where t_{ox} indicated the length of the oxidation reaction. *OTR* was then determined by:

$$OTR = C_{Na2SO3} \cdot \nu_{O2} \;/\; t_{ox} \qquad (1)$$

where C_{Na2SO3} and ν_{O2} are molar sodium sulfite concentration and stoichiometric coefficient for oxygen, respectively. K_La was calculated from the following relation provided by Hermann *et al*., (2001):

$$OTR = K_La\, L_{O2}\, p_{gO} \qquad (2)$$

where the oxygen solubility L_{O2} was 9×10^{-9} mol/(L・Pa) and the partial oxygen pressure in the gas phase p_{gO} was 0.2095×10^{5} Pa, if the air in the vessel was exchanged sufficiently.

The solution consists of 0.5M sodiumsulfite (99% purity, Wako Pure Chemical Industries, Ltd.), 10^{-7}M cobaltsulfate (99% purity, Wako Pure Chemical Industries, Ltd.) as a catalyst, 0.012M Na_2HPO_4/NaH_2PO_4 phosphate buffer (99% purity, Wako Pure Chemical Industries, Ltd.) and 2.4×10^{-5}M bromothymol blue (Wako Pure Chemical Industries, Ltd.) in deionized water. pH was adjusted to 8.0 by addition of 30% sulfuric acid. Before and during the preparation of the sodiumsulfite solution, the deionized water was gassed thoroughly with nitrogen gas to avoid a prior oxidation of the sulfite. The bromothymol blue as pH indicator shows a color change from dark blue above pH 7.3 to yellow at pH 6.2. The experimental setup was taken by a video camera (3CCD, DCR-TRV900, SONY Corp.), which gave the time t_{ox} through the internal clock. From Eqs.(1) and (2) with the above experimental condition, K_La can be obtained from the following equation;

$$K_La = C_{Na2SO3} \cdot \nu_{O2} \,/\, (t_{ox}\; L_{O2}\, p_{gO}) \qquad (3)$$

where C_{Na2SO3} =0.5 mol/L, ν_{O2} =0.5.

1.2 Comparison of optical method with ordinary method

To compare the optical method with the ordinary method, the volumetric mass transfer coefficient K_La was initially measured with the ordinary method proposed in the previous paper (Kato *et al.,*1997). The dissolved oxygen in the liquid was purged with nitrogen gas, then the oxygen concentration change due to the oxygen transfer from the liquid surface was measured with a polarographic digital dissolved oxygen meter (DO-715K, Bionics Instrument Co. Ltd.). The K_La was obtained from the following equation:

$$K_La = -\ln(1-C_b/C_s)/t \tag{4}$$

In this case, the oxygen concentration in the liquid was changed so slowly with time that the response delay of the cell (OX-16K, Bionics Instrument Co., Japan) was neglected.

K_La of the large cylindrical flask (D = 12, 14, 17 cm , H/D = 0.5,1.0 , d = 2－4 cm , N = 140－180 rpm) measured by the optical method was compared with K_La measured by the ordinary method. The difference between them was about 30% in the experimental range of K_La, 5×10^{-3} s^{-1} to 3×10^{-2} s^{-1}. To estimate the experimental error between the methods, K_La of the electrolyte solution was measured by the ordinary method. The effect of electrolyte in the water on K_La was not observed (D = 12 cm , H/D = 0.5 , d = 4cm , N = 120－200 rpm). Therefore the difference between the observed values of K_La between the two methods seems to be independent of liquid properties, for example the density and existing some electrolytes.

2. Results and Discussion

2.1 Effect of liquid film formed on flask surface

The effect of the wettability of flask wall on *OTR* was examined. Condition of water film on the flask inner surface is schematically shown in **Figure 2**. By using the coating material, the liquid film was not formed on the flask inner surface. The observed *OTR* of the coating flask was compared with that of the normal flask in **Figure 3** for Erlenmeyer flasks. As shown in Figure 3, *OTR* of the coating flask was lower about 20% than that of the normal flask regardless of the geometry of flask. Therefore the wettability of flask surface is one of the important factors to increase the fermentation performances. It was considered that gas-liquid interface area on the flask inner surface was reduced by the coating materials. To obtain the large *OTR,* the condition of flask inner surface was very important.

2.2 Effect of baffle on oxygen transfer rate

As mentioned above, the liquid film on flask was very important factor to increase *OTR*, and it is considered that to increase the liquid film surface area is effective to obtain the high value of *OTR*. The effect of the baffle of flask on *OTR* was examined. The baffled flask had been developed by Garden, Jr.(1962). As shown in **Figure 4**, *OTR* of baffled flasks was four times larger than that of normal flasks. **Figure 5** is the photograph of behavior of the liquid in flask. It is considered that the reason of larger *OTR* was not only to increase the wet surface area on flask but also to entrap many bubbles in flask, as shown in Figure 5.

2.3 Development of cylindrical baffled flask and large vessel

We developed a new cylindrical flask and large vessel based on the geometry of baffled flask as shown in Figure 1. **Figure 6** shows the comparison of the particle suspension in a normal cylindrical vessel with that in a cylindrical vessel with plate type baffle. Plate type baffle, which is normally used in a turbulent agitated vessel with an impeller, was not effective for the shaking cylindrical vessel, as shown in Figure 6. To generate the rotational flow was very important to obtain good liquid mixing for the shaking vessel (Kato *et al*., 1995). However, when the four plate type baffles were used in the cylindrical vessel, the particles on the vessel bottom did not suspend, because the rotational flow was not generated in the vessel (Kato *et al.,* 1996).

Figure 7 shows the comparison of baffled cylindrical flask and vessel with normal cylindrical flask and vessel. It was found that the baffled cylindrical flask and vessel have about three times larger K_La than a normal flask and vessel. For example, **Figure 8** shows the photograph of behavior of the liquid in normal and baffled cylindrical vessels, many bubbles were entrapped and the liquid in the vessel was mixed well.

Conclusion

The gas-liquid volumetric coefficient of oxygen transfer rate in several kinds of shaking flasks was measured by an optical method based on sulfite oxidation. It was found that

1. the liquid film on the flask surface was very important factor to increase K_La,

2. the baffled flask was effective to obtain larger *OTR*, and
3. a new developed baffled cylindrical vessel has about three times larger K_La than a normal vessel.

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Nomenclature

C_{Na2SO3}	= molar sodium sulfite concentration	[mol/L]
D	= vessel diameter	[cm]
d	= shaking diameter	[cm]
d_i	=top diameter	[cm]
H	= liquid height	[cm]
K_La	= volumetric mass transfer coefficient	[s^{-1}]
L_{O2}	= oxygen solubility	[mol/(L・Pa)]
N	= shaking frequency	[rpm]
OTR	= oxygen transfer rate	[mol/(L・s)]
p_{g0}	= partial oxygen pressure in gas phase	[Pa]
t_{ox}	= reaction time for complete oxidation of sulfite	[s]
V_L	= liquid volume	[mL]
ν	= kinematic viscosity	[m^2/s]
ν_{O2}	= stoichiometric coefficient for oxygen	[-]

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Figure Caption

Fig.1 Dimensions of Erlenmeyer flask, baffled flask and new cylindrical baffled flask and large vessel

Fig.2 Comparison of wettability of flask surface between normal flask and coating flask

Fig.3 Effect of liquid film on *OTR*

Fig.4 Effect of baffle of flask on *OTR*

Fig.5 Comparison of baffled flask with normal flask (N = 180rpm, d = 4 cm)

Fig.6 Comparison of plate type baffled cylindrical vessel with normal cylindrical vessel (N = 130 rpm, D = 17 cm, d = 4 cm)

Fig.7 Comparison of baffled cylindrical flask and vessel with normal cylindrical flask and vessel

Fig.8 Comparison of baffled cylindrical vessel with normal cylindrical vessel (N = 180 rpm, d = 4 cm)

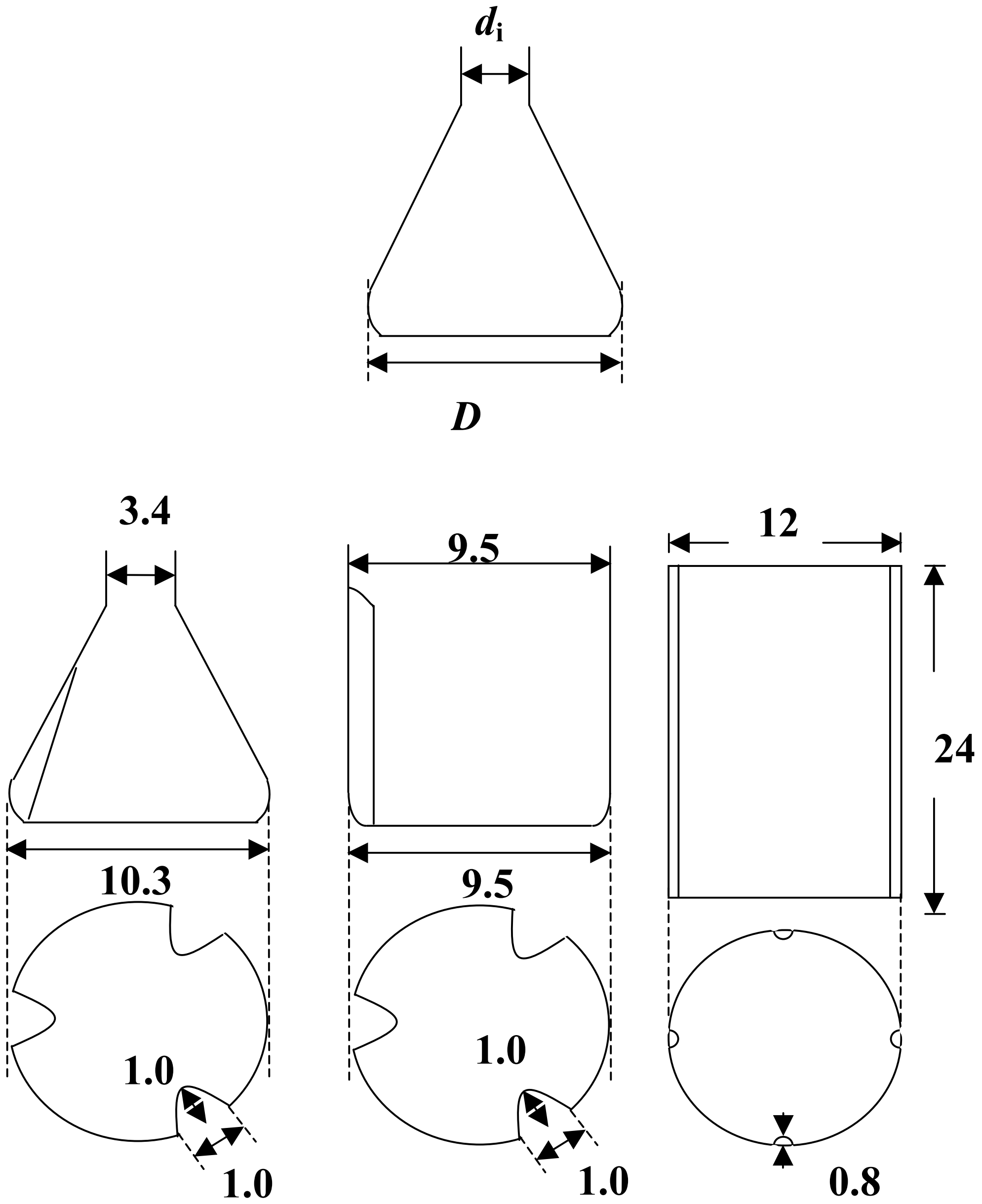


Fig. 1 Dimensions of Erlenmeyer flask, baffled flask and new cylindrical baffled flask and large vessel

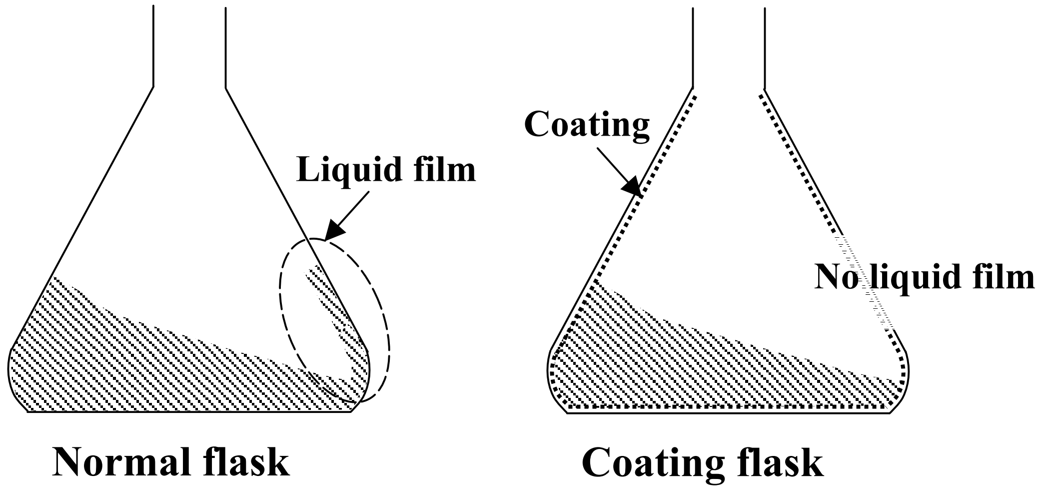


Fig. 2 Comparison of wettability of flask surface between normal flask and coating flask

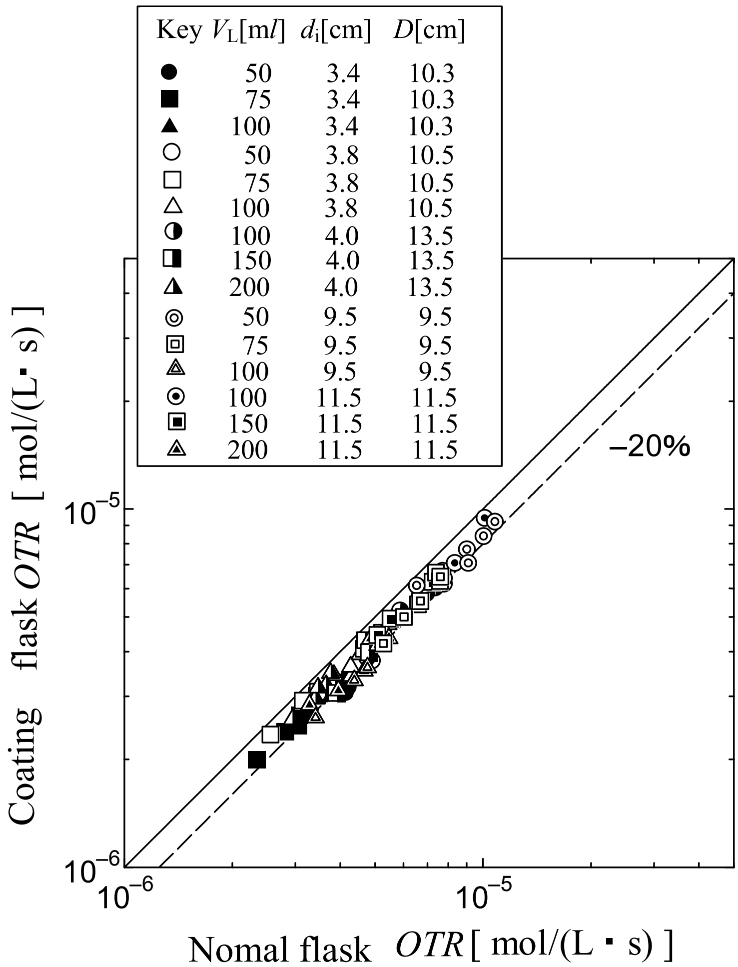


Fig. 3 Effect of liquid film on *OTR*

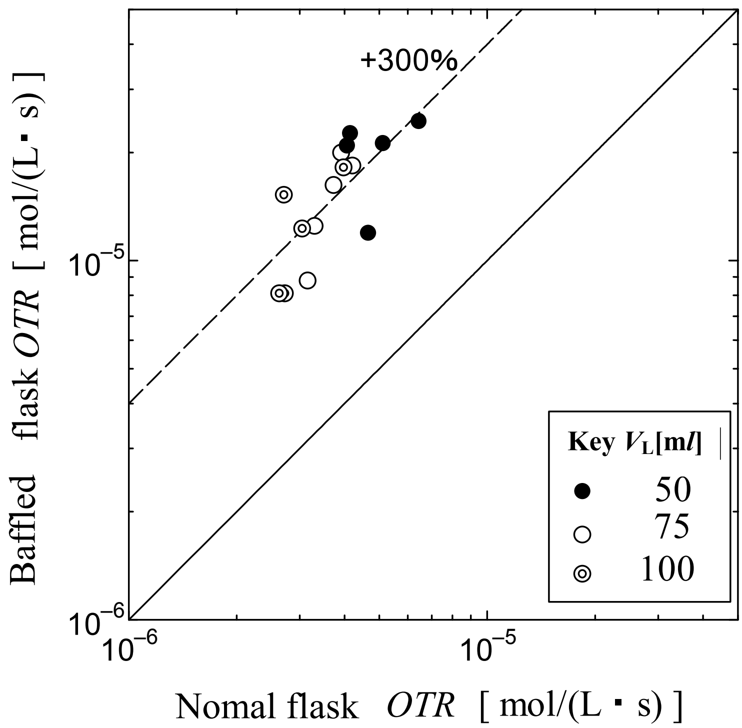


Fig. 4 Effect of baffle of flask on *OTR*

(a) Normal flask **(b) Baffled flask**

Fig. 5 Comparison of baffled flask with normal flask (N = 180 rpm, d = 4cm)

(a) Normal vessel **(b) Baffled vessel**

Fig. 6 Comparison of plate type baffled cylindrical vessel with normal cylindrical vessel
(N = 160 rpm, D = 15 cm, d = 4 cm)

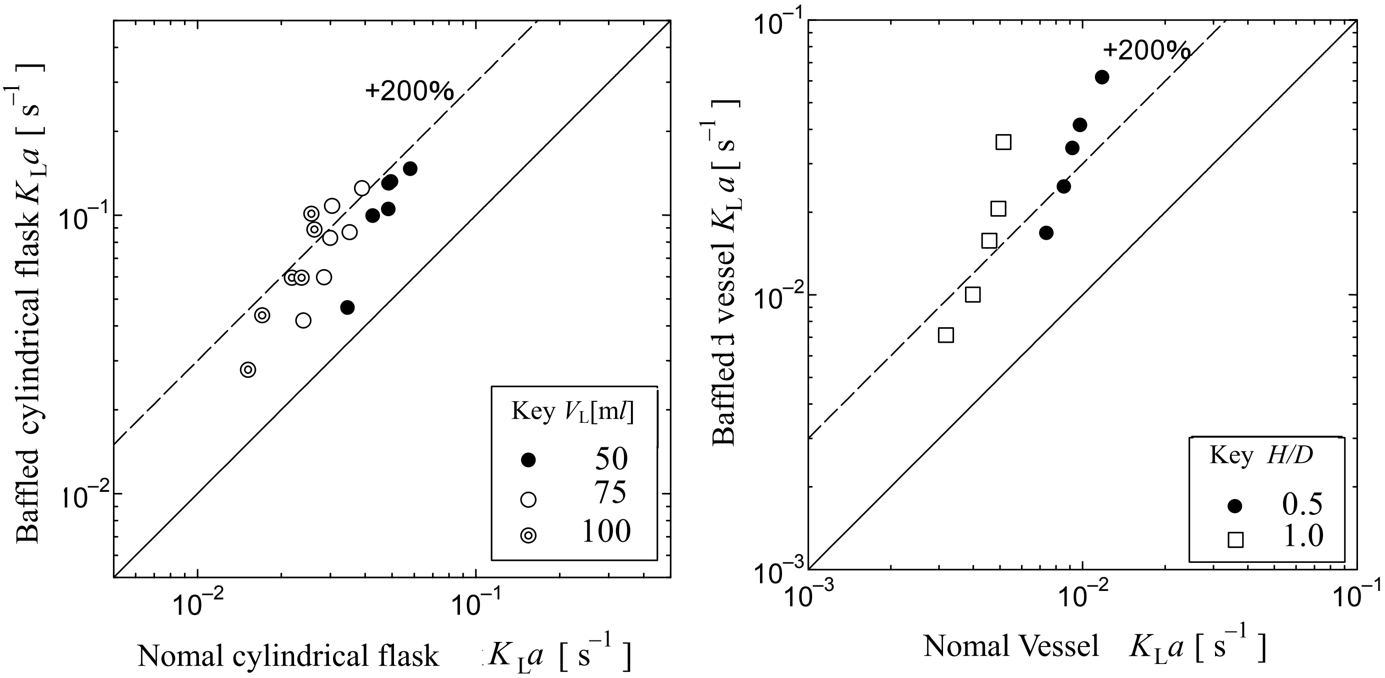
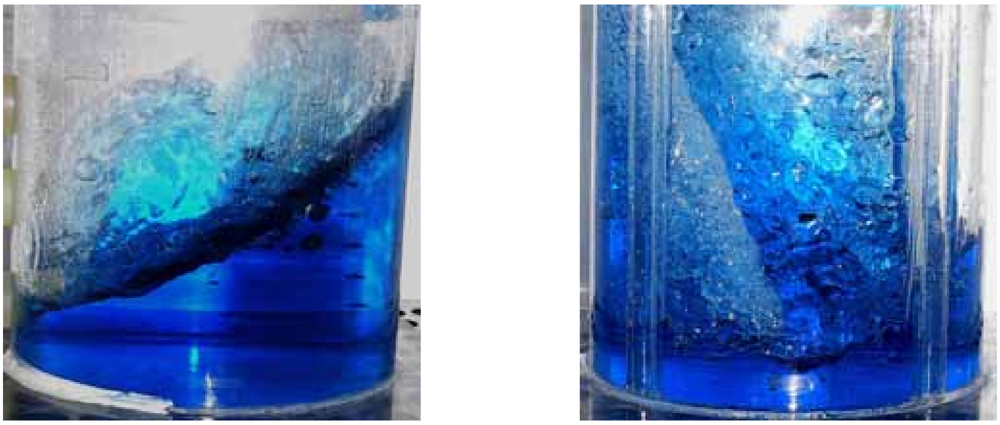


Fig. 7 Comparison of baffled cylindrical flask and vessel with normal cylindrical flask and vessel



(a) Normal vessel **(b) Baffled vessel**

Fig. 8 Comparison of baffled cylindrical vessel with normal cylindrical vessel (N=180rpm,d=4cm)