Enhancement of Oxygen Transfer by Pressure Pulsation in Aqueous Glycerol Fermentation

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Abstract

Aeration plays an important role in the production of glycerol by fermentation with yeast. Effective aeration depends on a number of factors, such as amount of air, fineness of air dispersion, rate of agitation, and time of gas-liquid contact. This investigation dealt with the effect of periodic variation in gas pressure on oxygen transfer measured by sulfite oxidation and glycerol fermentation in stirred tanks. The oxygen transfer rate measured with the sulfite oxidation method was improved by 20–30% under the condition of pressure pulsation (PP) at 30°C. The yield and productivity of glycerol were increased by about 26 and 6.8%, respectively, in 48 h by employing a glucose concentration of 250 g/L with PP at 30°C.

Index Entries: Glycerol fermentation; osmotolerant yeast; oxygen transfer; pressure pulsation; sulfite oxidation.

Introduction

Glycerol has wide applications in many chemical and plastic industries *(1)*. It can be produced through chemical or biochemical routes *(2)*. One of the biochemical processes is glycerol fermentation with osmotolerant yeast. Under hyperosmotic stress (high sugar concentration), the yeast cells must increase their internal osmolarity to retrieve water from the environment. The mechanism to survive is the accumulation of compatible solutes inside plasma membrane *(3)*; for instance, *Saccharomyces cerevisiae* uses glycerol as its sole compatible osmolyte. Glycerol production actually constitutes an important means to adapt to high external osmolarity.

Since the formation of glycerol is a part of the growth process of the osmophilic yeasts, productivity of glycerol can be influenced by the conditions of growth including the composition of the medium *(4)*, the concentration of dissolved oxygen (DO) *(5,6)*, pH value *(7)*, and temperature *(8)*.

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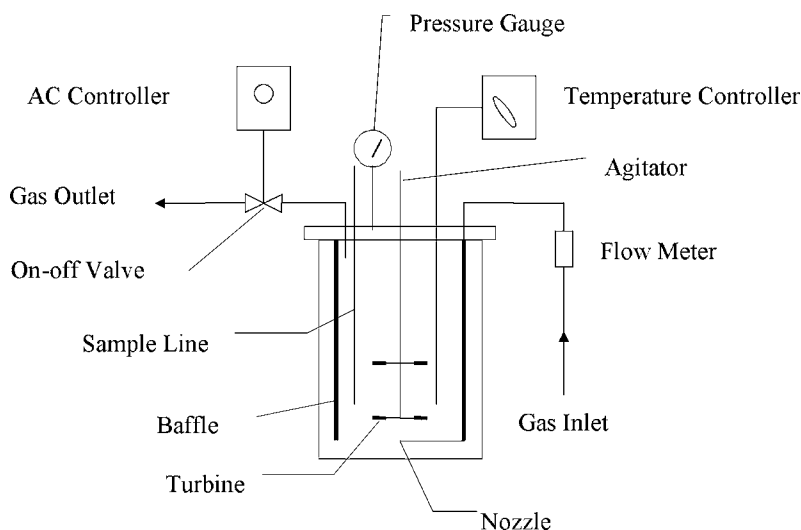
Fig. 1. Diagram of agitated-sparged fermentor with on-off valve on gas exit.

The oxygen-starved condition will shift the metabolic pathway toward ethanol formation. Namely, the production of glycerol by osmophilic yeasts is essentially an aerobic process in which the high oxygen transfer rate (OTR) is necessary. Under optimum condition of oxygen supply, glycerol formation can be maximized and ethanol production reduced to a negligible amount *(9)*. Yet, the means of regulatory control in the presence or absence of oxygen in the osmotolerant yeast remains unknown *(2)*.

We know that the effective aeration and high agitation rate can increase the OTR *(10,11)*, since these offer larger driving force, gas-liquid interfacial area, and longer residence time of gas bubbles in the liquid for oxygen transfer into the liquid. The aim of the present study was to find a more economical replacement to reduce the cost of compressed air and the power consumption of the agitator. The results showed that significant improvement in OTR measured by sulfite oxidation and the higher yield and productivity of glycerol were obtained with fermentation under pressure pulsation (PP) of 10 or 15 psig compared with that without PP. The effects of agitation and aeration were also examined.

Materials and Methods

Pressure Pulsation

PP is done by placing an on-off valve at the gas outlet of an enclosed reactor for the aerobic fermentation as shown in Fig. 1. When the exit valve is closed, the pressure in the vessel will build up owing to the accumulation of inflow; when the exit valve is open, the vessel depressurizes. This pressurization and depressurization repeated periodically is called as PP.

For example, by closing the outlet valve 8 s and opening it 2 s, each cycle will bring pressure in the enclosed vessel to pulsate between 0 and 10 psig with an average aeration rate of 1.7 vvm measured by wet test meter at 25°C and 1 atm. Similarly, closing the outlet valve 12 s and opening it 3 s periodically will bring 15 psig of PP.

Oxygen Transfer Rate

The liquid film surrounding the gas bubbles is usually the rate-limiting step for the oxygen transfer from gas bubbles to cells owing to the low solubility of gases *(12)*. The rate of oxygen transfer *(13)* from the gas to liquid phase is given by

$$\text{OTR} = \frac{dC_L}{dt} = k_L a \, (C^* - C_L)$$

where k_L is the oxygen transfer coefficient, a is the gas-liquid interfacial area, C^* is the saturated DO concentration, and C_L is the actual DO concentration in the broth.

Measurement of Sulfite Oxidation

In the presence of Cu^{2+} as the catalyst, sulfite is oxidized to sulfate in a zero-order reaction shown in the following equations *(14)*. Because of the rapid reaction, the actual DO concentration in the broth approaches zero. The OTR is the same as the rate of oxygen consumption, which is half of the rate of sulfate formation:

$$SO_3^{2-} + \frac{1}{2} O_2 \xrightarrow{\ Cu^{2+}\ } SO_4^{2-}$$
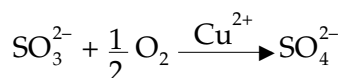
$$\text{OTR} = k_L a = \frac{1}{2} \frac{dC_{SO_4^{2-}}}{dt}$$

In a 1.5 or 1.8 L fermentor, 0.2 mol/L Na_2SO_3 and 0.63 mol/L $CuSO_4$ were added. Under different aeration rates, agitation speeds, and a PP of 10 or 15 psig, the OTR was studied at 30°C. A sample of 5 mL sulfite solution taken from the fermentor at different intervals was mixed with 5 mL of 0.2 N KI-I_2 solution. Excess I_2 was titrated with the 0.1 mol/L standard thiosulfate ($Na_2S_2O_3$) solution. The rate of the molar change of $Na_2S_2O_3$ solution used for the titration of two consecutive samples is the fourth of the OTR.

Yeast

All investigations for glycerol fermentation were carried out with osmotolerant yeast ICM-Y-05 (*Candida krusei*) from China. The stock cultures were maintained on slants with 100% YMP (3 g/L of yeast extract, 3 g/L of malt extract, 5 g/L of peptone), 30 g/L of glucose, and 15 g/L of agar.

Preparation of Inoculum

The culture from the stock agar slant was transferred into four 250-mL flasks with 100 mL of seed medium. The composition of the medium was 3 g/L of yeast extract, 3 g/L of malt extract, 3 g/L of peptone, and 15 g/L of glucose. The inoculum preparation was conducted on a rotary shaker at 160 rpm at 25°C for 48 h.

Fermentation

Cells from the preculture (about 0.5–1.0 g dry wt/L) were inoculated in the medium with a 1.8-L working volume. The fermentation medium consisted of the following: 250 g/L of glucose, 1.7 g/L of yeast extract, 3.3 g/L of urea, 3.3 g/L of KH_2PO_4, and 11.5 g/L of corn steep liquor. One milliliter of antifoam was added to prevent overflow of foam. The temperature of the fermentation was controlled at 30°C, the aeration rate was maintained at 1.7 vvm, and the agitator speed was kept at 500 or 700 rpm. At specified time intervals, 5 mL of sample was drawn aseptically, and after removal of the yeast cells by centrifugation, the clear fermented broth was subjected to analysis for residual concentration of glucose, glycerol, and ethanol.

Analytical Methods

An isocratic high-performance liquid chromatography (HPLC) system (AS-4000; Hitachi) equipped with an Aminex HPX-87H column (Bio-Rad, Melville, NY) was used to determine the concentration of glucose, glycerol, and ethanol. The mobile phase was 0.01 N H_2SO_4 at a column temperature of 60°C and a flow rate of 0.8 mL/min.

The dry weights of yeasts were obtained after centrifugation and drying in an oven at 120°C. The average density of yeast in broth is about 0.24 g dry wt/mL *(15)* measured at 72 h, the end of fermentation.

Results

Effect of Aeration Rate and Agitator Speed on OTR

Figure 2 shows that increasing the aeration rate only improved the OTR (measured by sulfite oxidation method) slightly; the higher the speed of the agitator, the larger OTR we can reach. This happens because (1) high-speed agitation can chop up the bubbles into smaller size, leading to larger interfacial area (gas dispersion); (2) the turbulent flow around the bubbles reduces the thickness of liquid film; and (3) the swift-eddy (a term used by R. K. Finn) *(10)*, which was caused by the high-speed agitation and resulted in a prolonged random motion path in the liquid, delays the escape of bubbles and lengthens the time of gas-liquid contact.

Effect of PP on OTR

The OTRs at various agitator speeds without PP are compared with those with a PP of 10 and 15 psig (at an aeration rate of 2.0 and 1.7 vvm,
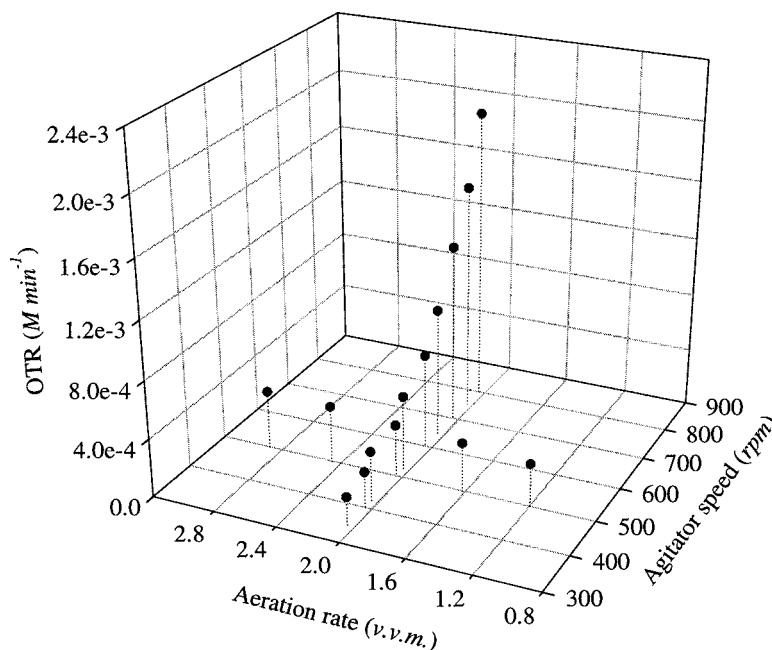
Fig. 2. Effect of aeration rate and agitator speed on OTR.

Table 1
Effect of PP (10 psig) on Sulfite Oxidation at Different
Agitator Speeds with Aeration Rate of 2.0 vvm

Agitator speed (rpm)	No PP	10 psig PP	Difference (%)[a]
400	3.2E-04[b]	3.3E-04	1.9
500	4.6E-04	5.9E-04	29
600	8.7E-04	9.8E-04	12
700	1.7E-03	2.1E-03	26
800	2.6E-03	3.3E-03	24

[a]Percentage difference: $(OTR_{w/PP} - OTR_{w/o\,pp})/OTR_{w/o\,pp}$.

[b]OTR (molar/min) measured at 30°C with working volume of 1.5 L.

working volume of 1.5 and 1.8 L, and 30°C), and percentage differences of OTR between that without PP and with a PP of 10 or 15 psig, are calculated, respectively, in Tables 1 and 2.

The application of PP on the sulfite oxidation system resulted in on average 22 and 37% higher OTR from bubbles to liquid phase at agitator speeds of 500 and 700 rpm, respectively.

Generally, if the agitator has a higher rotating speed, the PP system gives a larger increment of OTR. For example, with an aeration rate of 2.0 vvm under 10 psig of PP, the 29% improvement of OTR was achieved at an agitator speed of 500 rpm; with an aeration rate of 1.7 vvm under

Table 2
Effect of PP (15 psig) on Sulfite Oxidation at Different
Agitator Speeds with Aeration Rate of 1.7 vvm

Agitator speed (rpm)	No PP	10 psig PP	Difference (%)[a]
400	2.2E-04[b]	2.7E-04	25
500	3.5E-04	4.0E-04	14
600	6.0E-04	6.2E-04	2.8
700	8.5E-03	1.3E-03	48
800	1.7E-03	2.0E-03	19

[a]Percentage difference: $(OTR_{w/PP} - OTR_{w/o\,pp})/OTR_{w/o\,pp}$.

[b]OTR (molar/min) measured at 30°C with working volume of 1.8 L.

15 psig of PP, 48% improvement of OTR was reached at an agitator speed of 700 rpm. That is why the agitation speed at 500 and 700 rpm for glycerol fermentation was chosen.

Effect of PP on Glycerol Fermentation

To increase glycerol production, the PP technique was studied, and the results are shown as the Figs. 3–6 including the standard error bar calculated from four runs. The yield, productivity, yeast cell mass, and productivity per cell mass of glycerol production based on the glucose consumption are given in Tables 3 and 4. Tables 5 and 6 summarize the percentage differences of the cell mass, yield, and productivity of glycerol between fermentation with or without PP at different times at agitator speeds of 500 or 700 rpm and aeration rate of 1.7 vvm, 30°C.

Compared with the fermentation with PP, the uptake rate of glucose by yeast was 21% higher for that without PP after 24 h. The glycerol concentration was lower without PP, and it decreased after 60 h. This may result from the consumption of glycerol by yeast after the shortage of carbon source. The concentrations of cell mass with PP were larger than without PP after 36 h. Less ethanol was collected for batches with PP after 24 h. Either the transient depressurization accelerated the vaporization of ethanol from the gas headspace or the metabolic pathway shifted to the production of glycerol instead of ethanol.

Comparisons of yield, productivity, cell mass, and productivity per cell mass of glycerol production among the system without PP, with 10 psig, and 15 psig of PP at 24, 48, and 72 h are shown in Table 5. The data were the average of four repeated batches at an agitator speed of 500 rpm. The yield of glycerol decreased with time for the fermentation without PP, increased with time for that under 15 psig of PP, and had the maximum of 21% at 48 h under 10 psig of PP. All runs had maximum productivity at 48 h, and fermentation with PP of 15 psig showed higher productivity (0.67 g/[L·h]) than the other two systems at 72 h. More yeast cell mass was produced under 10 psig of PP compared with the other two cases at 24 and
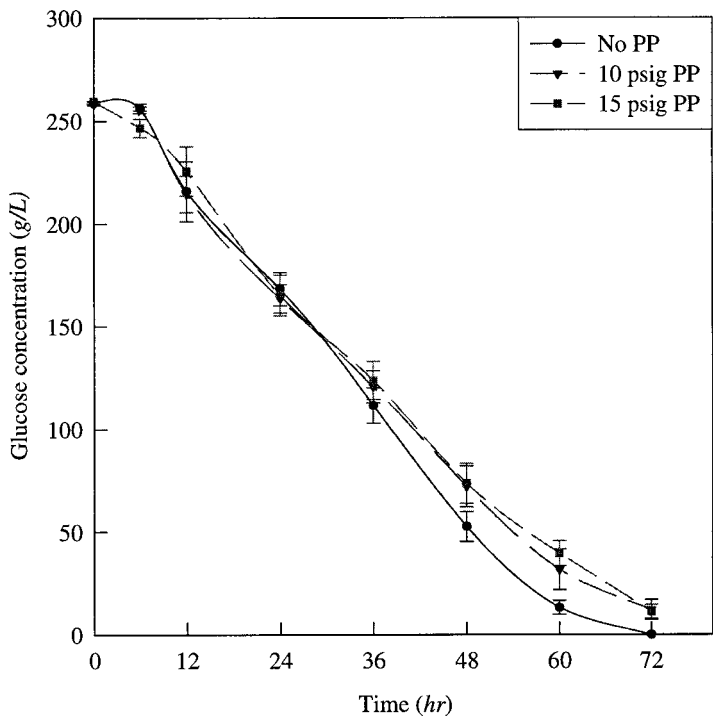
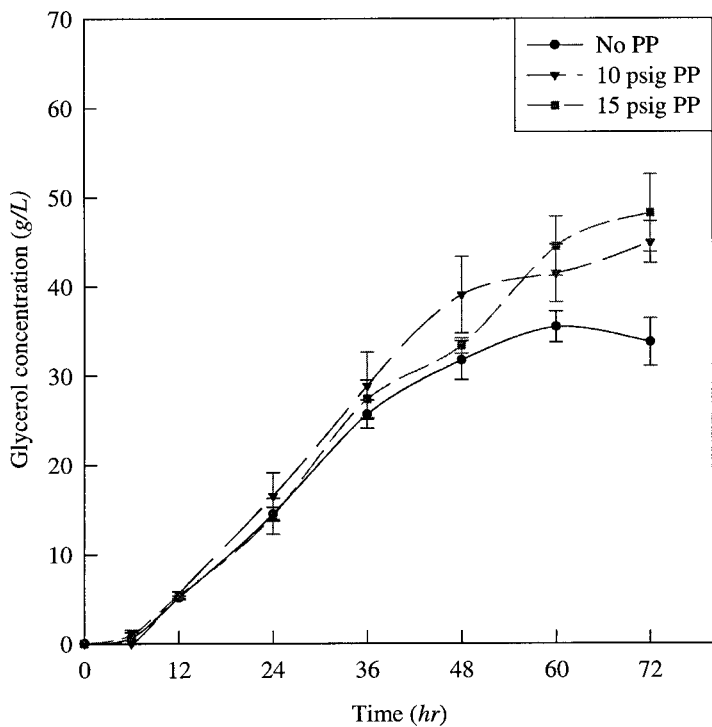Fig. 3. Effect of PP on glucose consumption at 500 rpm.

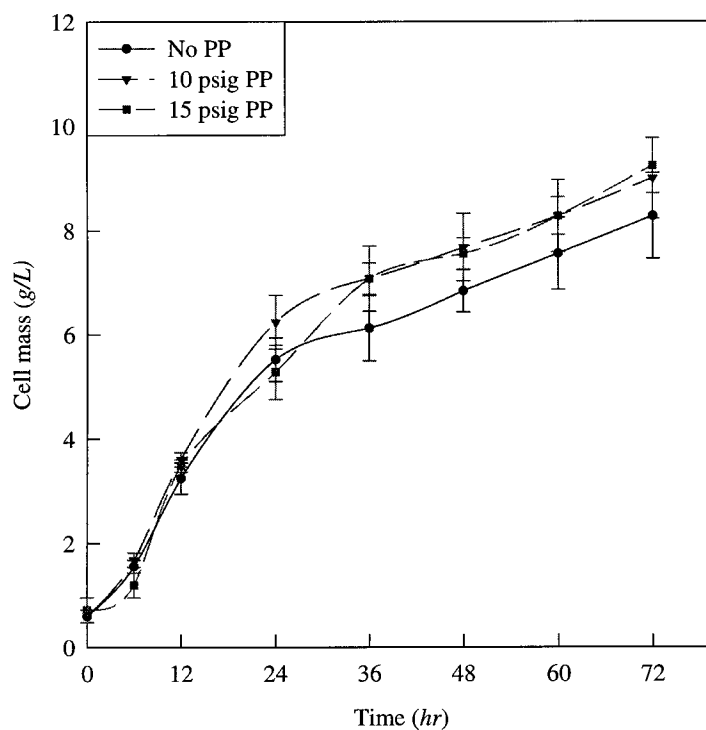Fig. 4. Effect of PP on glycerol production at 500 rpm.

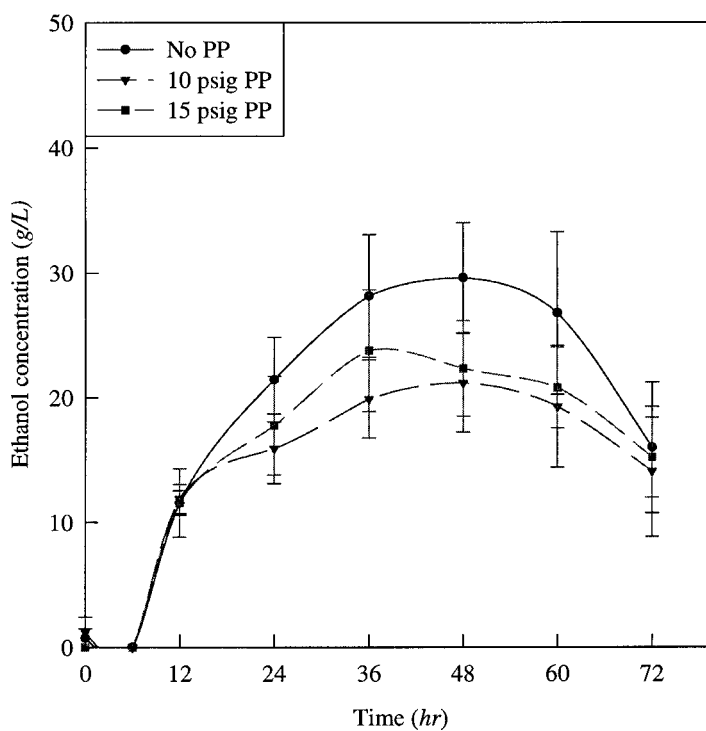Fig. 5. Effect of PP on yeast growth at 500 rpm.

Fig. 6. Effect of PP on ethanol formation at 500 rpm.

Table 3
Effect of PP on Cell Mass, Yield, Productivity, and
Productivity per Cell Mass of Glycerol Production at Different Times
with Agitator Speed of 500 rpm and Aeration Rate of 1.7 vvm at 30°C

Time	Yield × 100 (g/g)[a,b]			P × 100 (g/[L·h])[b,c]			Cell Mass (g dry wt/L)[b]			P × 100/cell mass (g/[g dw·h])[b]		
(h)	A	B	C	A	B	C	A	B	C	A	B	C
24	17	17	16	60	69	60	5.5	6.2	5.3	11	11	11
48	16	21	18	66	81	69	6.8	7.7	7.6	9.7	11	9.2
72	13	18	19	47	62	67	8.3	9.0	9.2	5.9	7.0	7.3

[a]Yield = mass of glycerol formed per mass of glucose consumed.
[b]A, no PP; B, 10 psig PP; 15 psig PP.
[c]P, productivity.

Table 4
Effect of PP on Cell Mass, Yield, Productivity, and
Productivity per Cell Mass of Glycerol Production at Different Times
with Agitator Speed of 700 rpm and Aeration Rate of 1.7 vvm at 30°C

Time	Yield × 100 (g/g)[a,b]			P × 100 (g/[L·h])[b,c]			Cell Mass (g dry wt/L)[b]			P × 100/cell mass (g/[g dw·h])[b]		
(h)	A	B	C	A	B	C	A	B	C	A	B	C
24	34	31	47	93	93	110	8.4	7.4	7.0	11	13	16
48	39	53	47	150	140	150	9.8	7.9	9.4	15	18	16
72	37	51	48	130	140	140	10	8.4	10	13	18	14

[a]Yield = mass of glycerol formed per mass of glucose consumed.
[b]A, no PP; B, 10 psig PP; 15 psig PP.
[c]P, productivity.

Table 5
Percentage Differences of Cell Mass, Yield, Productivity,
and Productivity per Cell Mass of Glycerol Between Fermentation
with or without PP at Different Times
with Agitator Speed of 500 rpm and Aeration Rate of 1.7 vvm at 30°C

Time	Difference of Yield (%)[a,b]		Difference of P (%)[b,c]		Difference of cell mass (%)[b]		Difference of P /cell mass (%)[b]	
(h)	A	B	A	B	A	B	A	B
24	4.1	−4.0	14	−1.5	13	−4.3	−2.3	0.29
48	35	17	23	5.1	12	11	8.5	−5.1

[a]Yield = mass of glycerol formed per mass of glucose consumed.
[b]A, 10 psig PP compared with no PP; B, 15 psig PP compared with no PP.
[c]P, productivity.

Table 6
Percentage Differences of Cell Mass, Yield, Productivity, and
Productivity per Cell Mass of Glycerol
Between Fermentation with or without PP at Different Times
with Agitator Speed of 700 rpm and Aeration Rate of 1.7 vvm at 30°C

Time	Difference of Yield (%)[a,b]		Difference of P (%)[b,c]		Difference of cell mass (%)[b]		Difference of P /cell mass (%)[b]	
(h)	A	B	A	B	A	B	A	B
24	−7.9	39	−0.10	22	−11	−17	14	47
48	35	19	−4.5	3.7	−2	−4.9	19	9.3

[a]Yield = mass of glycerol formed per mass of glucose consumed.
[b]A, 10 psig PP compared with no PP; B, 15 psig PP compared with no PP.
[c]P, productivity.

48 h. The most cell mass (9.2 g dry wt/L) was generated at 72 h under 15 psig of PP. The productivity per cell mass decreased as time passed. The longer the reaction time, the higher the productivity (compared with that without PP) for PP of higher-gage pressure.

The average yield of glycerol under 10 or 15 psig PP was 35 or 17% larger than one without PP, and the productivity was 23 or 5.1% larger in 48 h (Table 5). The average yield, productivity, cell mass, and productivity per cell mass of glycerol production were 26, 14, 12, and 1.7% higher than without PP in 48 h, respectively.

At an agitator speed of 700 rpm, Tables 4 and 6 show similar results to the system using an agitator speed of 500 rpm, with the following exceptions. The glycerol yield under 10 psig of PP reached the theoretical maximum of 52% after 48 h. The percentage differences of productivity at 48 h were much smaller than at an agitator speed of 500 rpm. The 10 psig of PP system almost prevailed over the 15 psig of PP system in the yield at 48 h. The percentage differences of cell mass decreased with PP, especially for the fermentation under 10 psig of PP at 48 h (−20%). The percentage differences of productivity per cell mass increased greatly at 48 h. Probably, the higher turbulence and pressure inhibited the cell growth, 7.9 compared with 9.8 g dry wt/L of that without PP.

The average yield of glycerol under 10 or 15 psig of PP was 35 or 19% larger than without PP, and the productivity was 4.5% smaller or 3.7% larger in 48 h (Table 6). The average yield, productivity per cell mass, productivity, and cell mass of glycerol production were 27, 14% higher, 0.4, and 13 lower than without PP in 48 h, respectively.

Discussion

The results have shown that under the PP of 10 or 15 psig, the OTR measured by sulfite oxidation improved by 20−30% on average compared

with the same condition without PP. The yield of glycerol obtained for fermentation under 10 and 15 psig of PP increased by an average of 26% in 48 h. The productivity of glycerol increased by 6.8% on average, and the productivity per cell mass increased by on average 7.9% in 48 h. The reasons that PP can improve the OTR are discussed next.

Enhancement of PP on OTR

For a single bubble, when the gas pressure changes from p_1 to p_2 by a factor of n,

$$p_2 = n\,p_1 \tag{1}$$

The bubble radius will change from r_1 to r_2. Assuming ideal gas and spherical bubbles, then

$$r_1^3 = n\,r_2^3 \tag{2}$$

$$a_1 = 4\,\pi\,r_1^2 = n^{2/3} a_2 \tag{3}$$

in which a_1 and a_2 are the surface area of the bubbles. Meanwhile, applying Henry's law ($HC^* = p$),

$$C_1^* = C_2^* / n \tag{4}$$

Equations 3 and 4 were substituted into the OTR equation described in Materails and Methods. Assuming that k_L is constant and C_L equals zero, we get

$$\mathrm{OTR}_1 = k_L a_1 C_1^* = k_L n^{2/3} a_2\, C_2^* / n = k_L a_2 C_2^*\!\left(n^{-1/3}\right) = n^{-1/3}\mathrm{OTR}_2 \tag{5}$$

In the case of 15 psig of PP, $n=2$, Eq. 5 is written as $\mathrm{OTR}_2 = 1.26\,\mathrm{OTR}_1$. Thus the OTR under 15 psig of PP will increase about 26%. This should be a major reason that PP indeed improves the OTR and the yield and productivity of glycerol production.

In addition, during the pressurization phase of the PP cycle, gas bubbles are compressed into smaller sizes. Generally speaking, a smaller bubble will ascend through the liquid slower owing to smaller buoyancy, and thus spend longer residence time in the broth to allow more of its oxygen content to be transferred into the liquid phase. By contrast, during the depressurization phase, the gas bubbles will expand quickly. This creates fresh bubble surface area. From the surface renewal model, fresh interfacial area has a much higher rate of oxygen transfer than the old bubble surface area. In addition, the PP creates constant contraction and expansion of the bubbles during their residency in the liquid phase. It creates a higher level of turbulence next to the bubble surface, which enhances the value of k_L by increasing the frequency of surface renewal. The enhancement of oxygen transfer from gas bubbles into surrounding liquid under PP should be the cumulative effects of all of these mechanisms. Studies of the detailed effects on k_L and interfacial area by PP are still under way.

PP can generate a substantial improvement in yield and productivity of about 20–30%. Meanwhile, lowering the agitator speed from 700 to 500 rpm can elevate the effects of PP from −0.8 to 14% for the productivity of glycerol in 48 h; even the yield and productivity at 500 rpm were less than half of that at 700 rpm. However, adding an on-off control valve on the gas exit line costs little compared with the price of compressed air and power consumption of a high-rotating agitator. Further investigation considering factors such as yield, productivity, and cost of compressed air and power consumption should be conducted before scale-up of the PP method.

Acknowledgments

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