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石油 炭化水素 醱酵에 미치는 攪拌의 効果

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The Effect of Agitation on Hydrocarbon Fermentation

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Abstract

요 약

石油炭化水素 醱酵에 있어 impeller의 크기와 攪拌速度가 攪拌効果에 미치는 影響을 數種의 flat-bladed turbine을 使用하여 究明하였다.

菌株로는 Rhodotorula種을 使用하였으며, 炭素數 12-14인 工業用 *n*-alkane을 炭素源으로 하여 28°C, pH 4.0~4.5 酸素供給速度 0.4 v.v.m. (Volume per volume per minute)의 條件下에서 醱酵를 進行하였다.

菌體收率, lag phase 및 generaition time은 impeller의 크기 및 攪拌速度에 의해 決定되는 攪拌所要動力에 직접 影響을 받았으며, 같은 動力入量에 對하여generaition time에 미치는 攪拌所要動力의 影響은 impeller의 直徑이 큰 경우가 impeller blade 面積이 넓은 경우보다 効果的 이었다.

Abstract

The effects of agitation depending on impeller geometry and agitation velocity in hydrocarbon fermentation have been studied for flat-bladed turbines in laboratory scale fermentor.

The microorganisms of Rhodotorula species were grown on *n*-alkane mixture of which carbon number ranged from 12 to 14 at 28°C, with pH range of 4.0-4.5 and at the oxygen flow rate of 0.4 of v.v.m. (vol./vol./min) in this experiment.

Cell yield, lag phase and generation time were influenced directly by power input in agitation which was determined by impeller geometry and agitation velocity for a given medium.

Even at the same power input the generation time was influenced more effectively by increasing impeller diameter than increasing blade area.

Introduction

Agitation effect on many aspects of submerged fermentation processes has been studied widely. Most of the experiments were concerned with two-phase system fermentation using water-soluble substrate, [1-3] and mass transfer in two-phase system was also investigated. [4-7]

Since the microorganisms are grown in water-insoluble medium, different from the common two-phase fermentation, it is necessary to disperse the hydrocarbon in water and enlarge the surface area of hydrocarbon particles in the hydrocarbon fermentation so that hydrocarbon can be easily attacked by the

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microorganisms.

Studies on oxygen transfer employing various flat-bladed turbine impellers showed significant differences between the two-phase and three-phase systems.[8-10]

Agitation effect on hydrocarbon fermentation with impellers of various geometry and at various agitation velocity is very important problem in the sense of engineering but little work has been reported on this subject.

In this experiment, the effect of impeller geometry and agitation velocity on hydrocarbon fermentation was studied using *n*-alkane mixture as carbon source and employing two series of flat-bladed turbine impellers.

Experiment

Microorganisms. Yeasts of Rhodotorula species isolated from oil-soaked soil were used in this experiment.

Medium. The medium for seed culture and main fermentation contained 4.7 g. KH_2PO_4, 4.0 g. NH_4NO_3, 0.3 g, Na_2HPO_4. $12H_2O$, 1.0 g, $MgSO_4 \cdot 7H_2O$, 0.01 g, $FeSO_4 \cdot 7H_2O$, 0.01 g, $MnSO_4 \cdot nH_2O$, 0.01 g, Na_2MoO_4, 0.01 g, $CaCl_2 \cdot 2H_2O$, trace of yeast extract and 10 ml hydrocarbon per liter of medium.

The hydrocarbon used as carbon source in this experiment was technical grade *n*-alkane mixture from Mitsubish Organic Chemicals. Its compositions analyzed by Vapor Fractometer were 19.3 percent of dodecane, 63.9 percent of tridecane and 16.8 percent of tetradecane and its specific gravity was 0.754.

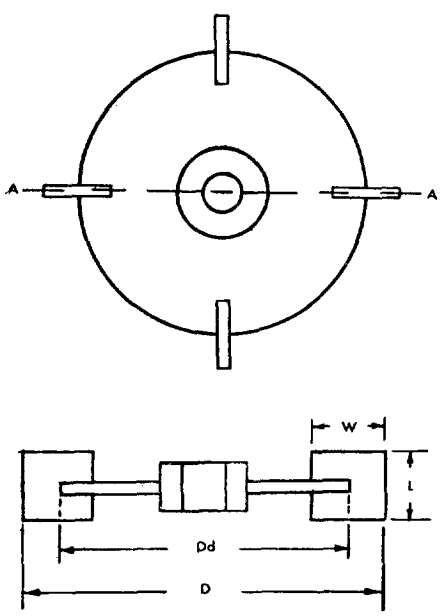

Fig. 1. Shape of Impeller.

Impellers. Main fermentation was carried out in a 2-liter fermentor of which inner diameter and hight were 120 mm and 220 mm, respectively. Two series of flat-bladed turbine impellers, as shown in Table 1 and Fig. 1, were used for agitator. In A series the overall diameter of the impeller was kept constant (6.0 cm) while varying blade dimensions considerably. The other series had constant blade dimensions with 4 standard blades (12×12 mm). The power inputs were measured with a dynamometer during fermentation.

Table 1. Impeller Dimension

Impeller	Overall Dia	Blade Height	Blade Width	Disc Dia.	Impeller Factor
	D(cm)	L(cm)	W(cm)	Dd(cm)	WI (D -W)
A—1	6.0	0.8	0.8	5.0	3.32
A—2	6.0	1.2	1.2	4.8	6.91
A—3	6.0	1.8	1.8	4.0	13.6
B—1	4.0	1.2	1.2	3.0	4,03
B—2	6.0	1.2	1.2	4.8	6.91
B—3	7.5	1.2	1.2	6.3	9.07

Fermentation. Seed culture for the main fermentation was prepared by inoculating 5 ml of the shaking culture to the 100 ml of medium in 1-liter Erlenmeyer flask sterilized by steam under 15 psig for 15 minutes and cultivating it in the Shaking Incubator at 28° C for 2 days.

One liter of the sterilized medium and seed culture were put into the sterilized fermentor. The ratio of seed culture to medium was 1/10. Fermentation of each batch was carried out at 28°C, with pH range of 4.0-4.5, at oxygen flow rate of 0.4 v.v.m. and at agitation velocity of 600, 1,000 and 1,500 r.p.m. for each impeller.

After fermentation, the microbial cells were harvested directly by centrifugation at 4,000 r.p.m. for 15 minutes and then washed with distilled water. The yields were expressed at percentage of the amount of dried cells per amount of hydrocarbon added.

Growth Rate. The logarithm of the cumulative volume of ammonium hydroxide consumption was plotted against fermentation time as shown in Fig. 2.

Ammonium hydroxide consumption was observed to be increased nearly exponentially with time throughout the fermentation. The slope of the resulting line indicates the exponential growth rate.[12]

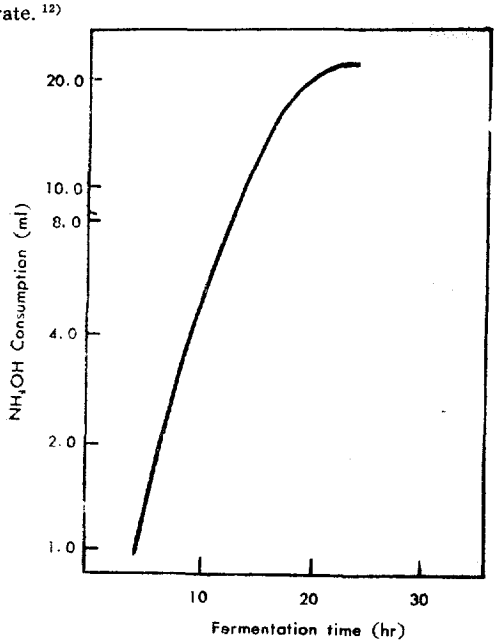

Fig. 2. NH_4OH Addition on Fermentation Time. (By Using B-3 Type Impeller, 1,500 r.p.m.)

Result And Discussion

The mechanical power absorbed by fermenting broth was measured for various impellers. These curves indicated in Fig. 3 show the linear relationship between the power input and agitation velocity for each impeller. No substantial differences have been noted during fermentation.

There were significant differences in the courses and results of fermentations for each impeller when fermentations were carried out at different velocities of 600, 1,000 and 1,500 r.p.m.

When the power input in agitation was small, there was air flooding in the beginning of fermentation because of insufficient agitation and there came severe foaming during fermentation as cells increased.

No foaming was seen during fermentation when

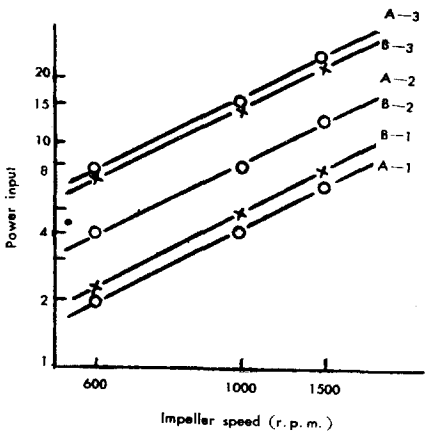

Fig. 3. The Effect of Speed & Size of Impeller on Power In put.

A-3 and B-3 Impellers, which were larger than the others in impeller size, were used and operated at the agitation velocity of 1,000 and 1,500 r.p.m. But there was a little foaming that could be controlled mechanically in case of A-2 and B-2 impellers operated at the agitation velocity of 1,500 r.p.m.

In connection with the power input which was directly related with agitation velocity and impeller size it could be said in this experiment that fermentation could be carried out with no foam if the power input in agitation was larger than the value of 12 watts per liter of medium.

Lag phase and generation time of microorganisms for each impeller operated at the agitation velocity of 600, 1,000 and 1,500 r.p.m. are presented in Table 2 and Table 3 respectively.

Table 2. Lag Phase (hr.)

Impeller / r.p.m.	A-1	A-2	A-3	B-1	B-2	B-3
600	—	11.7	9.5	—	11.7	7.2
1,000	11.5	7.5	5.2	12.3	7.5	5.2
1,500	5.5	5.3	5.0	7.5	5.3	4.7

In Table 2, it is found that lag phase was greatly influenced by agitation velocity and impeller geometry. With increasing of impeller size and agitation velocity, lag phase was shortened rapidly from 12 hours to 5 hours. In case of agitation velocity being 1,500

r. p. m. for all impellers except B-1 which was the smallest and 1,000 r. p. m. for A-3 and B-3 impellers, lag phases were about 5 hours constantly. On the contrary in case of agitation velocity being 600 r. p. m. for A-1 and B-1 impellers which were smaller ones, lag phases were so long that fermentation couldn't be carried out on economic basis. The relationship between lag phase and power input in agitation is presented in Fig. 4.

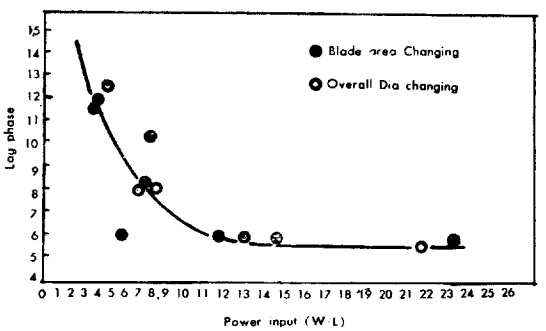

Fig. 4. Relationship between Power Input and Lag Phase.

When power input was larger than a certain value (12 watts/medium-liter), lag phase was about 5 hours irrespective of impeller size and agitation velocity, but below that value lag phase was increased randomly with power input decreasing. Therefore, it is necessary to ensure power input in agitation larger than that value to minimize the lag phase in hydrocarbon fermentation.

Table 3 shows that generation time was also influenced greatly by agitation velocity and impeller geometry as lag phase. With increasing of impeller size and agitation velocity generation time was shortened from 4.8 hours to 2.8 hours.

Table 3. Generation Time(hr.)

r. p. m. \ Impellers	A-1	A-2	A-3	B-1	B-2	B-3
600	—	4.6	4.2	—	4.6	4.1
1,000	4.5	3.9	3.3	4.8	3.9	3.1
1,500	3.9	3.4	3.1	4.0	3.4	2.8

The relationship between generation time and power input in agitation is presented in Fig. 5, where it is found that generation time was also decreased with power input increasing, but there were some different effects on generation time between the cases using impellers of different blade area and overall diameter.

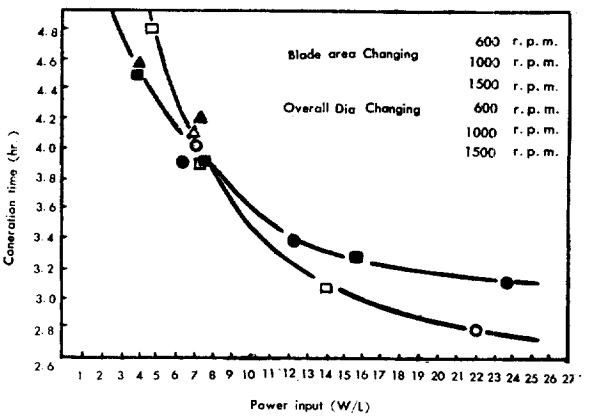

Fig. 5 Relationship between Power Input and Generation time.

The effect on generation time influenced by power input variation resulted from changing overall diameter with constant blade area seemed to be larger than that resulted from changing blade area with constant overall diameter within the scale of this experiment.

Even at the same agitation velocity, for the agitation of fermentor, to increase impeller diameter was more effective than to increase blade area of impeller and thus B-series gave shorter generation time than A-series when the value of power input was larger than certain value and below that value, A-series gave shorter generation time than B-series. For the agitation velocity of 1,000 r. p. m. it was 7.2 watts per liter of medium.

At the same power input, it is noted that an impeller of larger overall diameter and smaller blade area gave shorter generation time than an impeller of smaller overall diameter and larger blade area.

It is considered that at a given power input an impeller of larger overall diameter gives less flow and more shear in agitation than an impeller of

larger blade area.

From above consideration it seems that the growth rate of microorganisms may depen on the mode of power input as well as the rate of power input. Perhaps high peripheral speed at a given power input, especially considering in connection with air bubbling from the bottom of fermentor, is more effective than high flow in dispersing the fine hydrocarbon particles in water.

Resulting cell yields, as shown in Table 4, were ranged from 39. 5 % to 98. 2 % for various agitation conditions. It is found that cell yield was also greatly influenced by agitation velocity and impeller size. However, in the case of no foaming during fermentation and at lag phase of about 5 hours, high cell yields more than 80 % were obtained in this experiment.

Table 4. Cell yield

	A-1	A-2	A-3	B-1	B-2	B-3
600	—	39. 5	74. 2	—	39. 5	65. 5
1, 000	54. 1	70. 5	75. 4	39. 5	70. 5	90. 8
1, 500	75. 0	80. 7	91. 2	56. 9	80. 7	98. 2

Conclusion

Various significant results were obtained in hydrocarbon fermentation using water-insoluble n-alkane mixture as carbon source and turbine type impellers. The results can be summarized as follows:

1. For the value of power input for agitation larger than a certain value of 12 watts/liter of medium, there was no foaming during fermentation and the lag phase was about 5 hours irrespective of the impeller size and agitation velocity. While, below the certain value of the power input, the lag phase increased rapidly as the power input decreased.

2. Generation time was decreased from 4. 8 to 2. 8 hours with increasing impeller size and agitation velocity. Even at the same agitation velocity, the increase of the overall diameter of impeller gave larger effects than the increase of the blade area of impeller.

3. It was shown, in this experiment, that the optimum power input for agitation was 12 to 14 watts per liter of medium with respect to the lag phase and the generation time in the hydrocatbon fermentation.

4. Cell yields of yeast were ranged 40-98 percent for agitation conditions.

References

1. J. M. West and E. L. Gaden; Biotech. & Bioeng., **1**, 163(1959).
2. R. steel and W. D. Maxon; Biotech. & Bioeng., **4**, 231 (1962).
3. A. Virglio, E. Marcelli and A. Agrimino; Biotech. & Bioeng., **6**, 271 (1964).
4. W. D. Maxon; J. Biochem. Microbiol. Tech. Eng., **1**, 311 (1969).
5. C. H. Cooper, M. A. Fernstrom and S. A. Miller; *Ind. Eng. Chem.*, **36**, 504 (1944).
6. A. M. Friedman and B. N. Lightfoot; *Ind. Chem. Eng.*, **49**, 1227 (1957).
7. E. O. Karow, W. H. Bartholomew and M. R. Saft; *J. Agri. Food Chem.*, **1**, 302 (1953).
8. G. Hamer and N. Blakebrough; *J. Appl. Chem.*, **13**, 517 (1963).
9. N. Blakebrough and K. Sambamurthy; *J. Appl. Chem.*, **14**, 413(1964).
10. N. Blakebrough and K. Sambamurthy; Biotech. & Bioeng., **8**, 25 (1966).
11. T. W. Park; Report to Korea Oil Corporation on Petro-protein Production (II), (1970).
12. T. L. Miller and M. J. Johnson; Biotech. & Bioeng., **8**, 549 (1966).