Batch, Fed-batch, Semi-continuous Culture for Highly Viscous Polysaccharides Production

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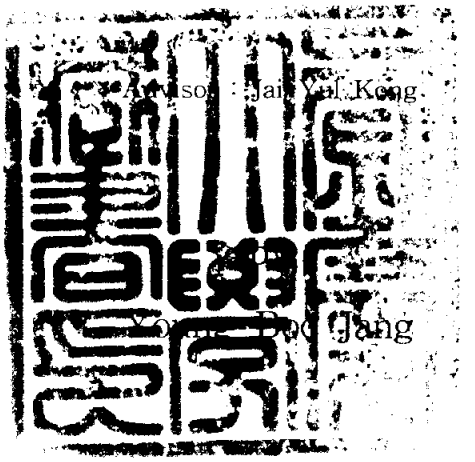

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Batch, Fed-batch, Semi-continuous Culture for
Highly Viscous Polysaccharides Production

A Dissertation

by

Young-Boo Jang

Approved as to style and content by :

Chairman Joong Kyun Kim

Member Jong Deog Kim

Member Jai Yul Kong

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Batch, Fed-batch, Semi-continuous Culture for Highly Viscous Polysaccharides Production

Young-Boo Jang

Department of Biotechnology and Bioengineering, Graduate School.

Pukyong National University

ABSTRACT

A newly isolated marine bacterium, identified as *Zoogloea* sp.(KCCM10036), produced two fundamentally different extracellular polysaccharides: Water-Soluble Polysaccharide(WSP) and Cell-Bound Polysaccharide(CBP). Especially, these polysaccharides showed a highly viscous and so its various applications for the industrial use are expected. So, We carried out the batch, fed-batch, semi-continuous culture for the polysaccharides production. The optimum nitrogen source and C/N ratio were bacto peptone and 5. The optimum of the batch cultures were a 3L working volume, 400 rpm, 1vvm, 30 ℃, 2.5 %(w/v) glucose as a carbon source, 10 % (v/v) inoculum size. These polysaccharides production was 11.3 g/ℓ after 120 h of cultivation. The optimal conditions of the fed-batch culture, feeding volume and concentration of feeding substrate were 200 mL and 2.5 %(v/v) glucose. At the feeding medium was supplied in an interval for 24h and the polysaccharides production was 6.28 g/L after 120 h of cultivation and the final volume of culture broth was 3.8 L. The semi-continuous culture of optimal conditions turned out with the 500 mL feeding volume, 10 %(v/v) inoculum size. At the feeding time interval was 24h that when it was condition as this, production of the polysaccharides was 8.67 g/L after 144h of cultivation and the final volume of culture broth was 8 L. We carried out the batch, fed-batch, semi-continuous culture for the polysaccharides production and its yield were 11.3, 6.28 and 8.67 g/L, respectively. However, productivity of the last polysaccharides of the batch, fed-batch and semi-continuous culture were 6.80, 4.77 and 9.75 g/L, respectively.

Consequently, the semi-continuous culture proved to be the most effective fermentation mode for the polysaccharides production from *Zoogloea* sp..

Key words: Highly viscous polysaccharide, *Zoogloea* sp., Batch, Fed-batch, Semi-continuous

Ⅰ. INTRODUCTION

As a magnitude of ocean resources was newly recognized recently, it was come to the front to the biotechnology field where marine biotechnology was new. Especially, marine microalgae and microbial faster than a large seaweed or marine animal growth and an artificial large culture is especially possible. It is comparatively easy to apply gene recombination and new biotechnology as protein engineering, and be suitable for compunction industrialization by production of biopolymer[1].

Also, they have a lot of kinds to have an useful biopolymer component, and usefulness is the highest among marine creatures[2]. A form environment maintained marine creature with land creature, and were adapted to an aquatic environment, and, as for the bioactive metabolite, a report became by having a case of land creature and other chemical structure. The study results that marine nature water was chemical, various menstruation activities of marine creature were informed, and started. Biotechnology technology is used by ocean creature in order to develop the resources which biopolymer applies a medicine, food to basic materials industries and used[1,3]. Bacterial surface polysaccharides come in two general forms, those bound to the cell surface by attachment to lipid. A lipopolysaccharide (LPS), and those associated with the cell surface as a capsule, exopolysaccharide (EPS)[4,5]. Also, polysaccharides is classified in according to specific electric charge, but the cationic polysaccharide which is the special polysaccharide that configuration amino sugar is becoming N-acetylation as polysacchaides including the dextran, leavan, pullan,

curdlan which is the homopolysaccharide that the anionic polysaccharide, that alginate, phosphomannan, xanthan gum was composed of layman natural polysaccharide and uronic acid is most natural polysaccharide, amino sugar[6].

Microbial exopolysaccharides such as xanthan and dextran have been commercial products for many years ; the search for new gelling agents has yielded gellan[7-9].

Zooglan can be used in non-food areas such as water clarification and paper finishing. *Zoogloea ramigera* as biosorbents for heavy metals offers a potential alternative to existing methods for detoxification and recovery for toxic or valuable metal from industrial discharge water[4,10].

Zoogloea sp.(KCCM10036) produced two fundamentally different extracellular polysaccharides : Water-Soluble Polysaccharide (WSP) and Cell-Bound Polysaccharide (CBP). These polysaccharides, WSP and CBP showed anti-tumor activity[11] and the useful characteristics of biopolymer as an effective adsorbent of heavy metal ions and as a new matrix for enzyme immobilization and the CBP gel beads showed highly effective adsorbing in Cr, Pb, and Fe ion in solutions[12-18]. Especially, these polysaccharides produced showed a high viscosity and so its various applications for the industrial use are expected[19,20]. Because density of polysaccharides accumulated in a culture broth of polysaccharide production is increased, a fluid characteristic of a culture broth changes into Non-Newtonian to Newtonian[21-24]. While bulk of macromixing region and stagnant region is increased in the existing fermentor of an agitation formula form in case of high viscosity culture broth relatively while there are a few bulks of

the micromixing region that high shear rate and an oxygen transfer are fluent in, as for the activity of the internal average cell which let a little of fermentor inside, be decreased to lack of nutritive substance[25].

Therefore, let you form an inequality stagnation of cells, and, as for the decrease of mass diffusion coefficient, an oxygen transfer decrease and the problem occur in the fermentor inside by gas transmissivity decrease with particular high viscosity in function polysaccharides fermentation[26,27].

Also, we carried out experimentation with the batch, fed-batch and semi-continuous culture with a way to solve the high viscosity problem of the polysaccharides production from *Zoogloea* sp.(KCCM10036).

Ⅱ. MATERIALS AND METHODS

1. Chemicals

All chemical were purchased from either (Difco Lab., Michigan, USA) or Sigma Chemicals(Sigma Chemicals Co., St. Louis, Mo., USA) and were of reagent grade[17,19].

2. Bacterial strain

Marine microorganism producing polysaccharides was isolated from the southern sea of Korea and this strain was identified as *Zoogloea* sp.(KCC-M10036). A loop of colony was transferred on slant and incubated at 30℃ for a day, and stored at 4℃[16].

3. Medium and cell culture

3-1. Medium

The composition of the basal medium for maintainable culture and fermentation was shown in Table 1[19].

3-2. Culture of microorganism

Flask cultures were grown in 500 mL baffle flasks containing 100 mL of medium on rotary shaking incubator at 180 rpm, 30℃, pH 7.8 and 1 %(w/v) glucose[17].

Table 1. Composition of modified marine medium

Component	Composition
Peptone	5g
NH_4NO_3	0.0016g
Na_2HPO_4	0.008g
Glucose	25g
Natural Sea Water	1L
pH	7.8

4. Flask Culture

4-1. Effect of nitrogen sources

To examine the effect of various nitrogen sources for cell growth and the polysaccharides production. *Zoogloea* sp. was incubated in medium containing various nitrogen source(Casamino acid, Urea, Thiourea, Beef extract, Gelatin, Yeast extract, Proteose peptone, Peptone G, Bacto soytone, Bacto pepton)[25,26].

4-2. Effect of C/N ratio

Generally, it is known that polysaccharides production was influenced by C/N (glucose / peptone) ratio. The C/N ratio optimal culture conditions were grown in 500 mL baffle flasks containing 100 mL of medium on rotary shaking incubator at 180 rpm, 30 ℃, pH 7.8, the C/N ratio ranging from 1 to 7 for 5 days[6,27].

5. Fermentation

5-1. Preculture for culture

In order to inoculum, single colony on agar plate was transferred to 500 ml baffle flask containing 100 mL of modified marine medium, and cultivated in a rotary shaking incubator at 30 °C, 1 %(w/v) glucose, 180 rpm, 18h, Then 10 % (v/v) culture broth was transferred to the fresh growth medium cultivated for 18h under same conditions. This seed was used to the inoculum of the fermentation.

5-2. Batch culture

The optimum of the batch cultures were a 3 L working volume, 400 rpm, 1 vvm, temperature at 30 ℃, 2.5 %(w/v) glucose as a carbon source, 10 % (v/v) inoculum size and then a medium in a fermentor was sterilized at 121 ℃ for 40 min[16,28].

5-3. Fed-batch culture

The polysaccharides produced by marine bacterium *Zoogloea* sp. in 7 L fermentor with the fed-batch culture. The fed-batch culture was conducted for feeding volume of 100, 200 and 500 mL, 3 L working volume and concentration of feeding substrate (% glucose, v/v) ; 2.5, 5.0, 10. Remainder conditions is same as batch culture. At the feeding medium was supplied in an interval for 24 h[29].

5-4. Semi-continuous culture

In this experiment, 7 L fermentor with the semi-continuous culture was used 5 L working volume. The feed indicated the time in which the medium has been replaced. The others conditions were the same with the batch and fed-batch, Executed experimentation on condition as inoculum size (%, v/v); 10, 20, and feeding volume of 200, 500 and 1000 mL. At the feeding time interval were 12 and 24 h.

6. Analysis

6-1. Isolation of polysaccharides

The culture broth was diluted with same volume of distilled water to reduce viscosity and mixed well with a magnetic stirrer.

It is isolate with crude WSP in distillation with the later polysaccharide which it got which washed a lot of order to the solution which winds up the polysaccharide which deals with cold (-20℃) acetone of 2 times, and came into being with a round glass rod, and was mixed after centrifugation in 4 ℃, 9,000 rpm by acetone and distillation 1:1 for 10min by supernatant for dissolved 12h after it accompanies a culture broth of 40 mL with number distillation of equivalence after a fermentation culture, and mixing. CBP puts number distillation with a cell sediment got by centrifugal disconnection in order to be same as the first bulk, and subsequent, small suspend deals with agitation in 60 ℃ intensely for 24h, and centrifugal later put cetylpyridinium chloride(CPC) with 10 % letting rear cool in 4 ℃ which separated for 9,000 rpm, 60 ℃, 10min with this, and it is isolate with polysaccharide. Freeze-drying did a dialysis thing for the later 12h that rear accompanied with cold acetone of 2 times, and separated only CBP that warmed different polysaccharide over 10 % NaCl solution 1 L, and separated CBP from CPC and got crude CBP, and a distillation washed pellet, and the cell got[30].

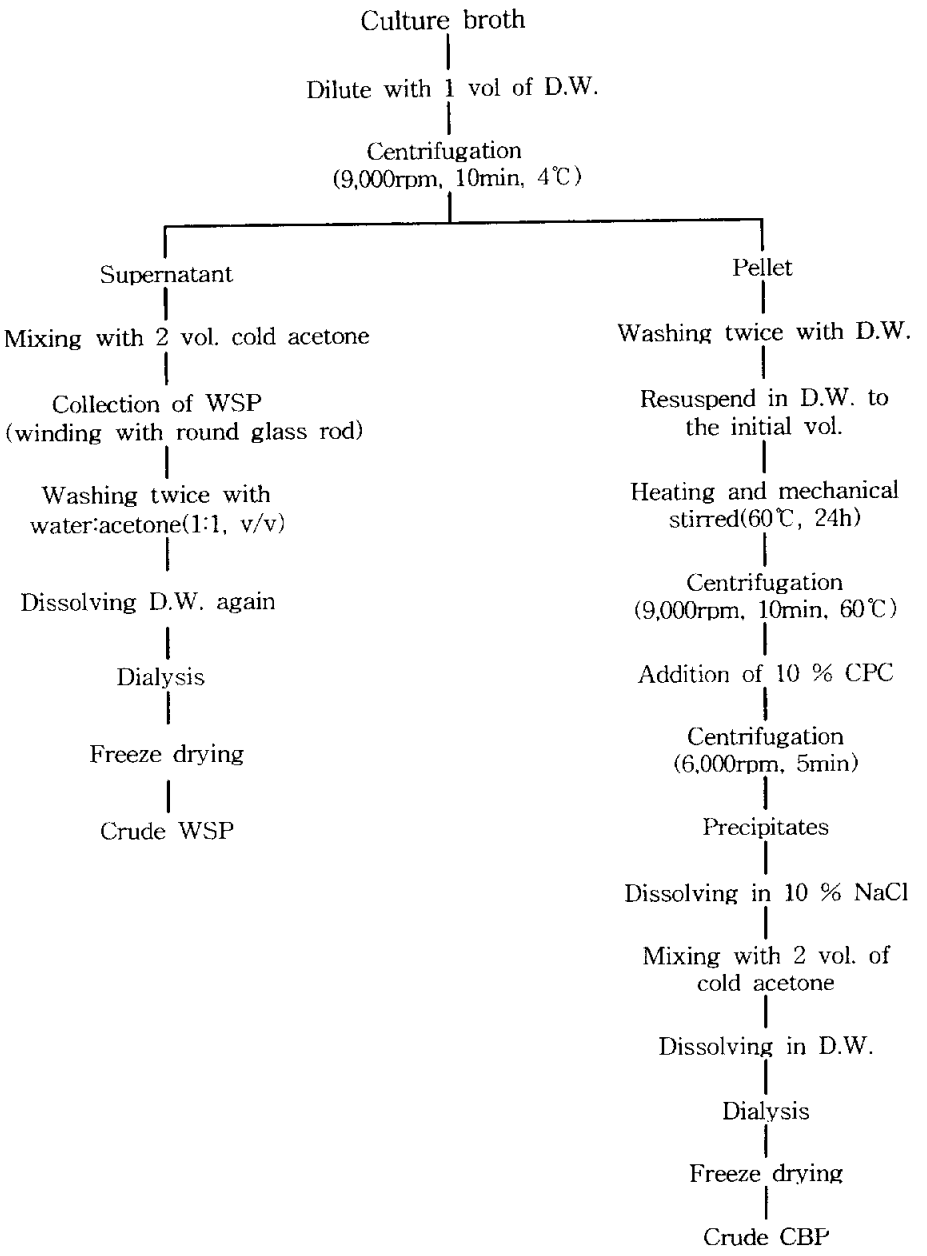


Fig. 1. Isolation steps of crude polysaccharides from culture broth.

6-2. Measurement of the polysaccharides production

As described at the front used foil weighed previously, and to have left that acetone precipitation was later in the isolation steps that went along and described and dried and rescued WSP, CBP and weight from dry-oven for 12 h and converted into 90 ℃ on 1 L and did a calculation[30].

6-3. Dry cell weight

Weight of cell used the foil which got overnight done by dry-oven and it was dry and measured weight of cell in 90 ℃ for 12h. The weight of cell converted comparison with the standard curve that dilution did supernatant of sample measured at 660nm with UV/VIS spectrophotometer (Ultrospec 3000, Pharmacia Biotech Ltd., England)[Fig.2][16].

6-4. Scanning electron microscopy (SEM)

In case of scanning electron microscopy (SEM) study, the cell was cut with liquid nitrogen and fixed in cold 2 % (w/v) glutaraldehyed at 4 ℃ for 2 h. After that the cell was washed with sterilized ethanol 30, 50, 80, 90, 95 % (v/v). And the cell was exchanged ethanol for anil acetate, and critical-point dried, sputter coated with gold, and examined with a scanning electron microscopy (Hitachi s-2400 SEM, Japan)[31].

6-5. Measurement of glucose consumption

Dilution did supernatant of 1mL and reacted with a DNS (3,5-Dinitrosalicylic acid) method[32] and glucose consumption measured at 550 nm with UV/VIS spectrophotometer and compared with glucose standard curve and measured remaining glucose[Fig.3].

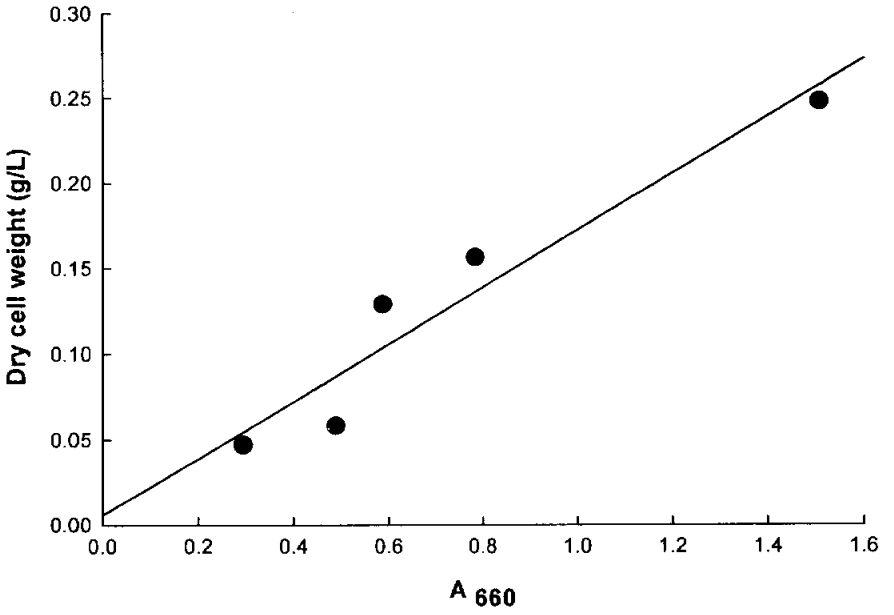


Fig. 2. Standard curve of dry cell weight by Absorbance.

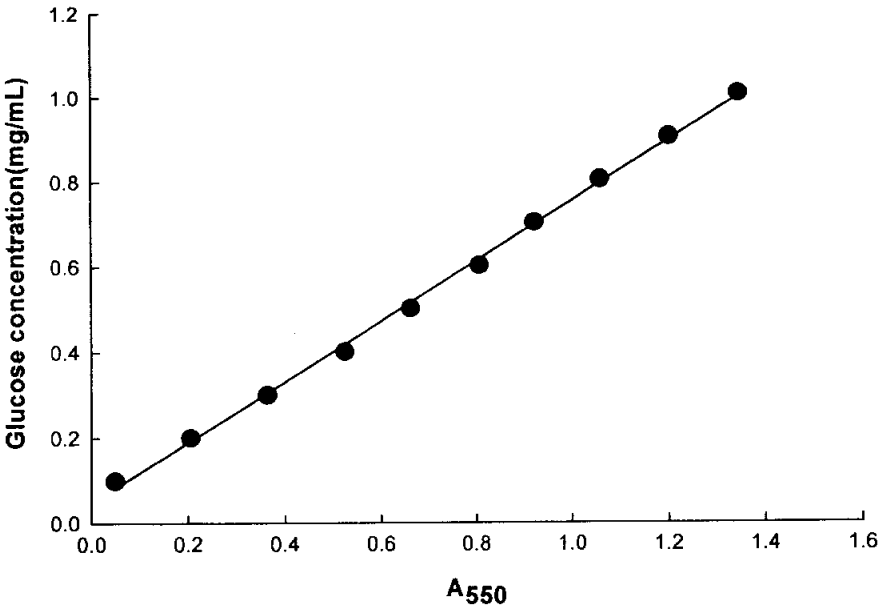


Fig. 3. Standard curve of glucose concentration by the DNS assay.

Ⅲ. RESULTS AND DISCUSSION

1. Characterization of isolated bacterium

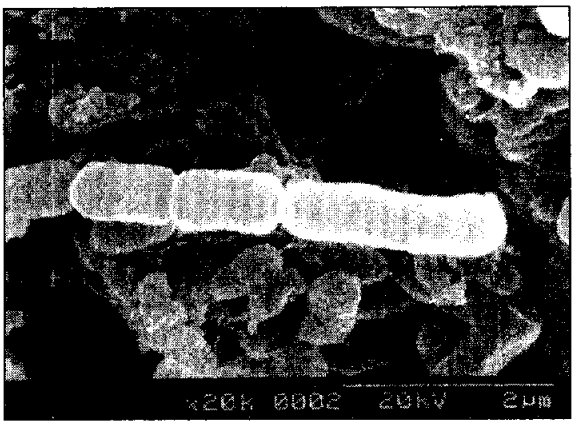
An extracellular polysaccharides producing bacterium *Zoogloea* sp.(KCCM10036) was isolated from marine environments. This strain could produce two different the polysaccharides. One (Water-Soluble Polysaccharide : WSP) was from cell-liquid medium, the other (Cell-Bound Polysaccharide : CBP) was obtained from cell surface. Both the polysaccharide productions started at the early stage of the logarithmic growth phase, the amount of WSP and CBP was influenced by culture conditions such as additional carbon and nitrogen sources. Isolated *Zoogloea* sp. show a high product yield without the increase of cell mass[17]. To results observed with SEM, straight to slightly curved, plump rods, 1.0-1.3㎛ in diameter and 2.1-3.6㎛ in length, with rounded ends [Fig.4]. Nonsporeforming and noncystforming. Cell in older cultures are demonstrably encapsulated[1,33,34].

2. Batch culture

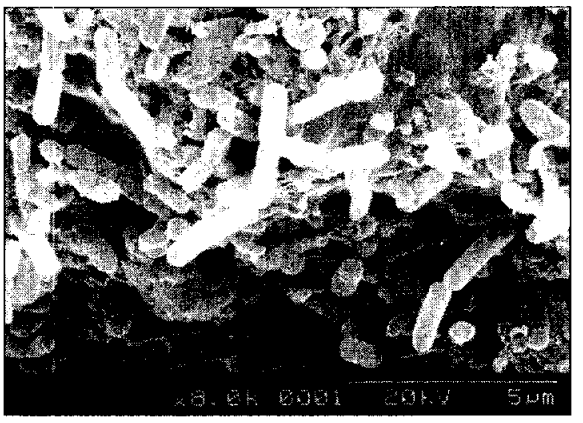
Executed a nitrogen source necessary and experimentation about C/N ratio in growth of cells in batch culture. Tested the nitrogen source in fermentation and flask, and the C/N ratio in flask.

2-1. Effect of nitrogen sources

The effect of nitrogen sources on the polysaccharides production was investigated in flask culture and batch culture. First of all, we carried out



a



b

Fig. 4. SEM of *Zoogloea* sp.(KCCM10036) on the batch fermentation with culture time (a, after 24h; b, after 48h).

complex nitrogen sources such as casamino acid, urea, thiourea, beef extract, gelatin, yeast extract, peptone G, proteose peptone, bacto soytone and bacto peptone in flask culture. A shown in Fig.5, bacto peptone was most effective for the polysaccharides production from various nitrogen sources, was 3.70 g/L. Therefore, the polysaccharides production was 11.3 g/L with what was produced in the medium which 0.5 %(w/v) accompanied with the results, the bacto peptone which determined four high rank nitrogen source (protease peptone, peptone G, Bacto soytone, Bacto peptone) with a nitrogen source of the batch culture which used fermentor by a foundation, and cultured this individually[Fig.6-9][33-35].

2-2. Effect of C/N ratio

The objective of the experiment is to determine the effect of the C/N ratio on the efficiency of the polysaccharides production in flask culture[16]. The polysaccharides production was increased in the range of 1-5, but results C/N ratio decreased from 6. The maximum production was the polysaccharides of 7.13 g/L which was C/N ratio 5[Table 2][8,11].

3. Fed-batch culture

In a fed-batch culture, the substrate concentration can be maintained at a fairly low level and the unfavorable effects of a high concentration, such as growth inhibition, can be avoided. The optimum feeding strategy of the substrate should be investigated so as to obtain the maximum production [9,33,36]. The fed-batch executed experimentation though the basic results

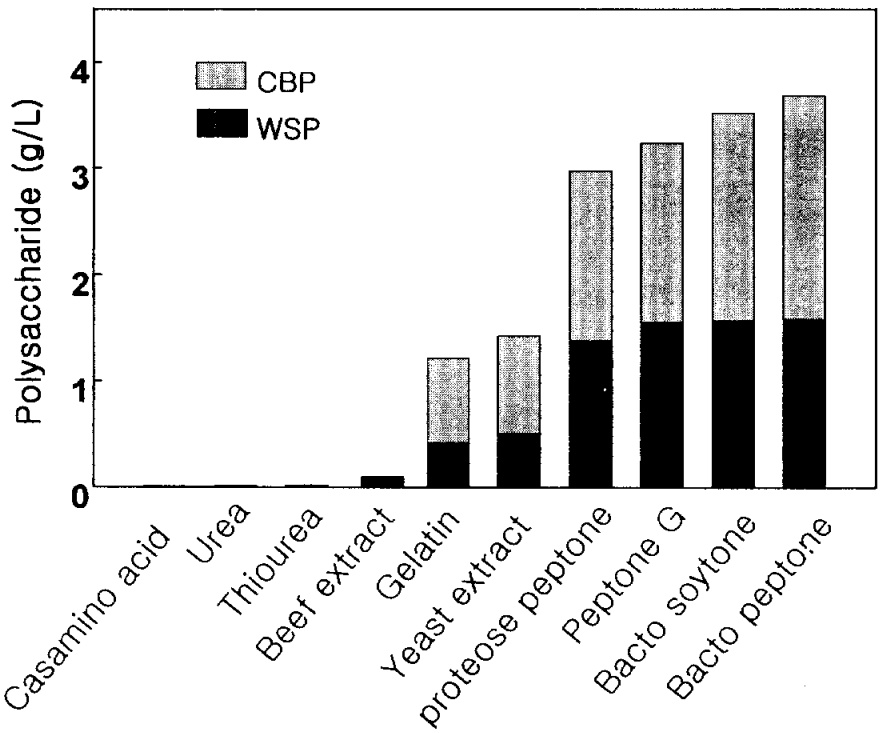


Fig. 5. Effect of the nitrogen source for the polysaccharides production.

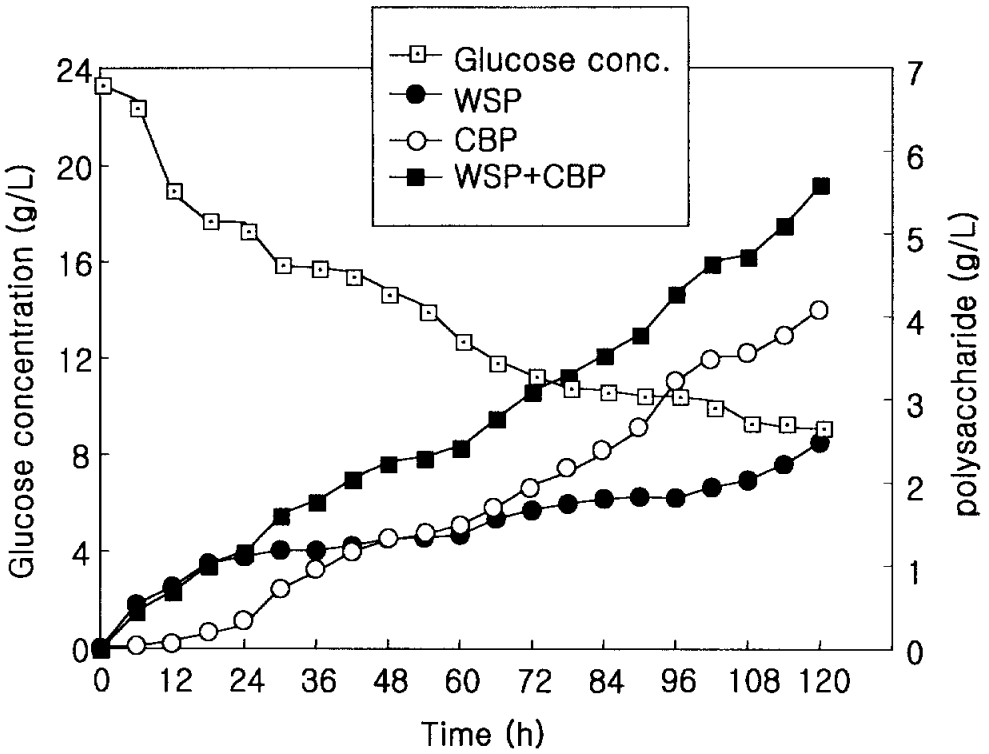


Fig. 6. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7L fermentor with the Batch culture 0.5% Bacto soyton, 2.5% Glucose, 400rpm, 1vvm, 10% inoculum size. The polysaccharides production was 5.61g/L.

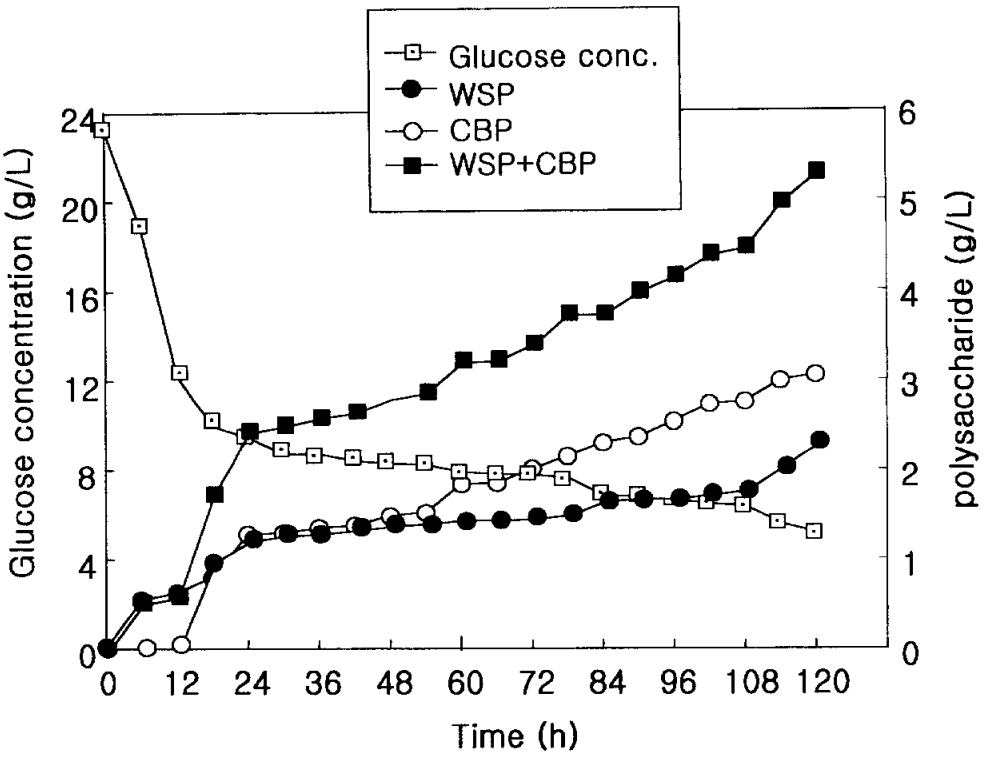


Fig. 7. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7L fermentor with the Batch culture 0.5% Protease peptone, 2.5% Glucose, 400rpm, 1vvm, 10% inoculum size. The polysaccharides production was 5.33g/L.

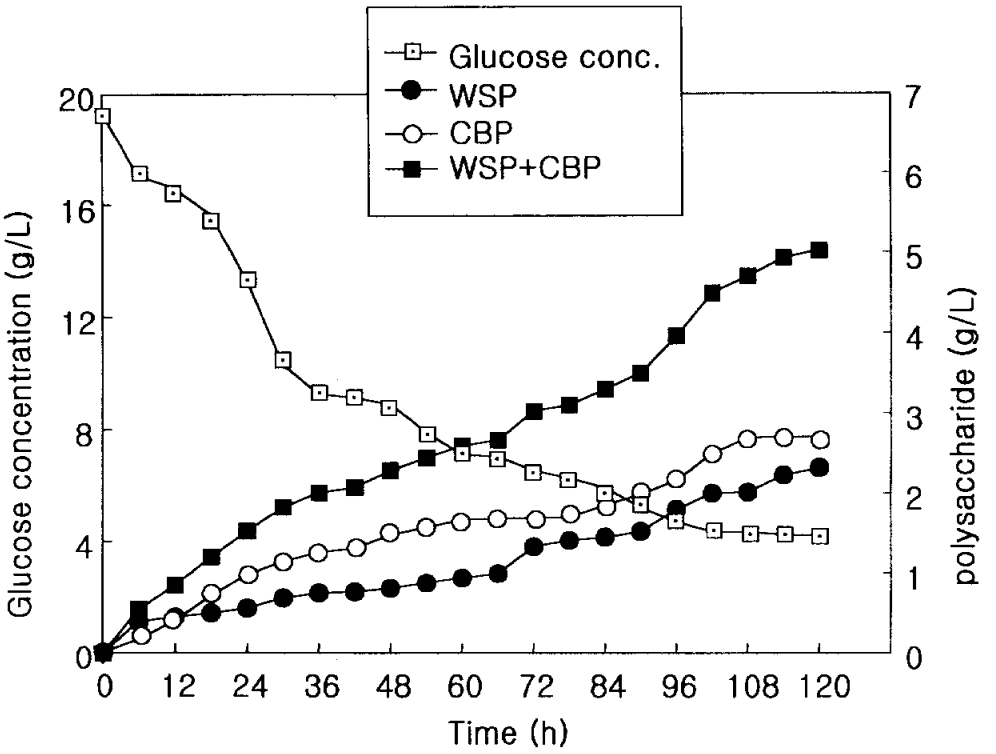


Fig. 8. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7L fermentor with the Batch culture 0.5% Peptone G, 2.5% Glucose, 400rpm, 1vvm, 10% inoculum size. The polysaccharides production was 5.02g/L.

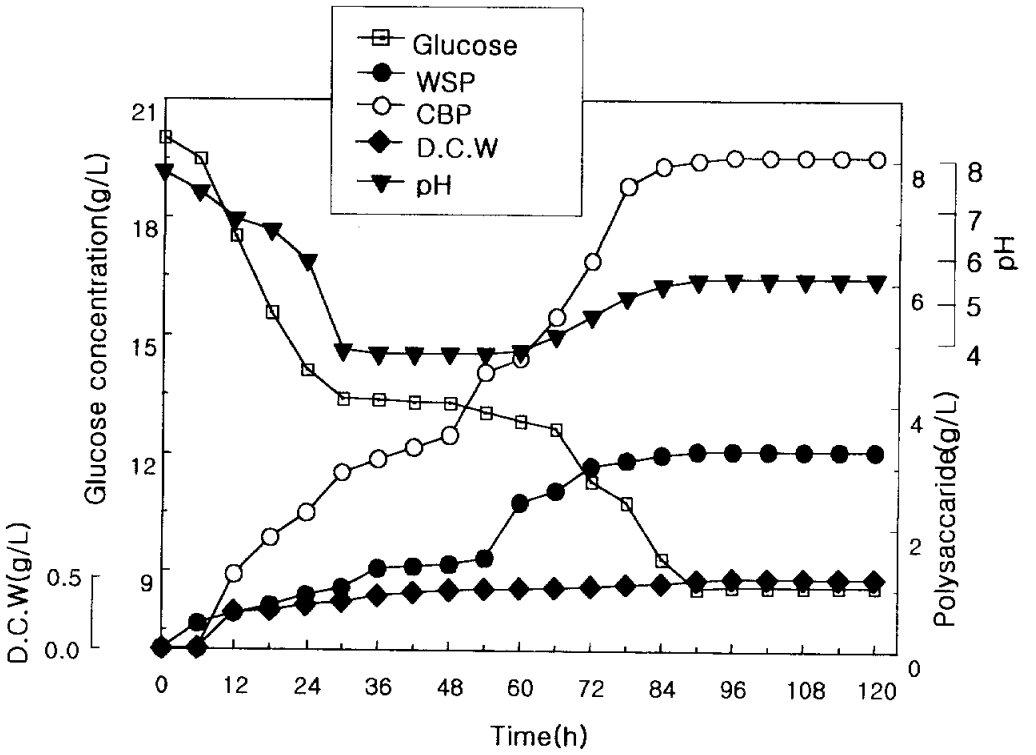


Fig. 9. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7L fermentor with the Batch culture 0.5% peptone, 2.5% Glucose, 400rpm, 1vvm, 10% inoculum size.

Table 2. Effect of the C/N ratio for the polysaccharide production

C/N ratio	Carbon source(g/L)	Nitrogen source(g/L)	Polysaccharide(g/L)		
			WSP	CBP	Total
1	0.5	0.5	3.487	0.787	4.274
2	1.0	0.5	2.063	3.508	5.571
3	1.5	0.5	2.405	3.530	5.935
4	2.0	0.5	2.166	4.081	6.247
5	2.5	0.5	2.409	4.719	7.128
6	3.0	0.5	2.507	1.712	4.219
7	3.5	0.5	2.991	0.218	3.209

got in batch. The experimentation carried out experimentation productivity of the polysaccharides though concentration of substrate and optimization of feeding volume in experimentation. Consequently, the optimal conditions feeding volume and concentration of feeding substrate were 200 mL and 2.5 %(v/v) glucose[37-40]. The polysaccharides production was 6.28 g/L after 120h of cultivation and the final volume of culture broth was 3.8 L.

3-1. Intermittent feeding conditions

3-1-1. Feeding volume

By the using a fed-batch culture with various pattern[Table 3] of feeding substrate, the result showed[Fig.10-12] in the polysaccharides production were 5.42, 6.28 and 4.76 g/L, respectively. Consequently, the optimum feeding volume was 200 mL.

3-1-2. Concentration of feeding substrate

The polysaccharides production from *Zoogloea* sp. in the fed-batch culture were executed with feeding concentration of optimal substrate in the range of 2.5, 5 and 10 % and experimented on the feeding volume with 200 mL. As the result showed a similar inclination by 24h, but excluded glucose of 2.5 % that supplied with feed, and appeared because polysaccharides production decreased. The production of polysaccharides rather decreased to substrate inhibition by excessive substrate concentration in glucose of 5 and 10 % (w/v)[Fig.13,14]. The polysaccharides production in concentration of feeding substrate were 6.28, 5.12 and 4.35 g/L, respectively. Consequently, the optimum concentration of feeding substrate was 2.5 %(w/v)[Fig.12].

Table 3. Effect of various feeding conditions for the polysaccharides production in the fed-batch culture

Feeding conditions		Cell concontration (g-D.C.W./L)	Polysaccharides Production(g/L)
Feeding volume (mL)	100	0.41	5.42
	200	0.54	6.28
	500	0.59	4.76
Concentration of feeding substrate (% glucose, v/v)	2.5	0.54	6.28
	5	0.32	5.12
	10	0.29	4.35

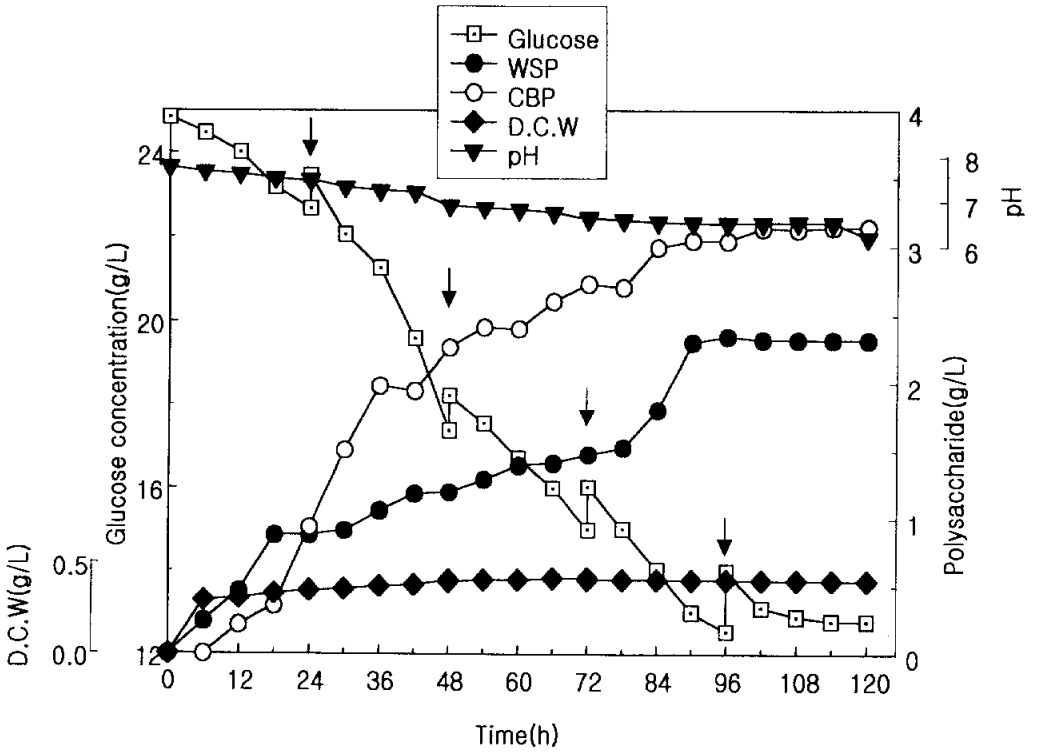


Fig. 10. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7 L fermentor with the Fed-batch culture, 2.5 % Glucose. The arrows(↓) indicated the start of medium feeding each other (2.5 g glucose/100 mL medium).

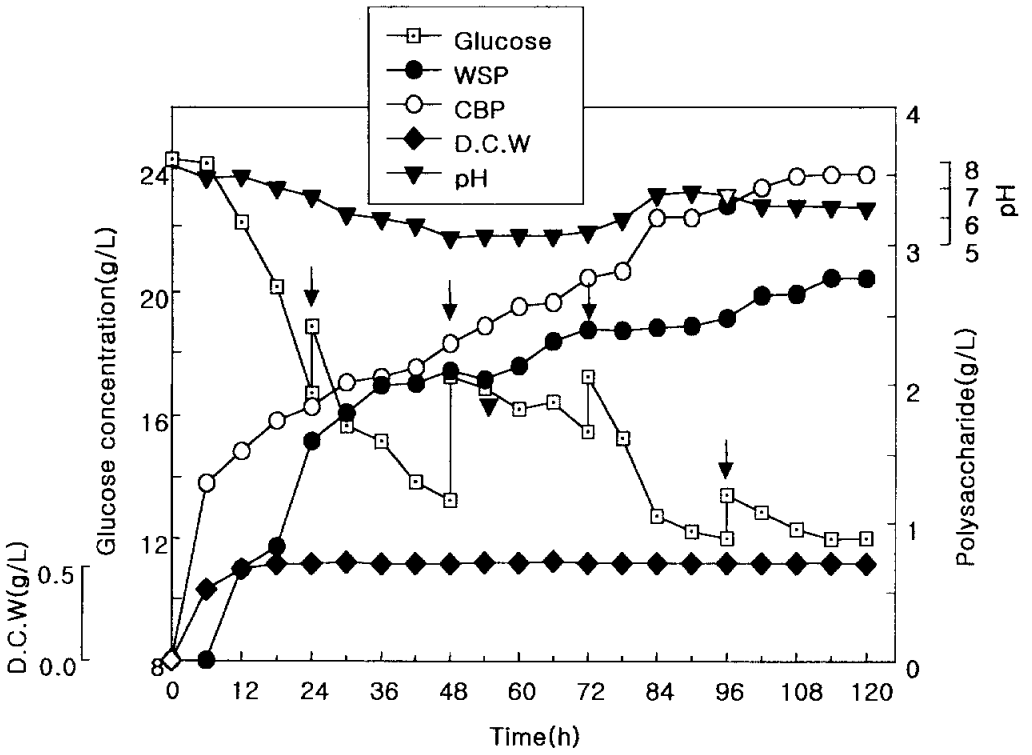


Fig. 11. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7L fermentor with the Fed-batch culture, 2.5 % Glucose. The arrows(↓) indicated the start of medium feeding each other (5 g glucose/200 mL medium).

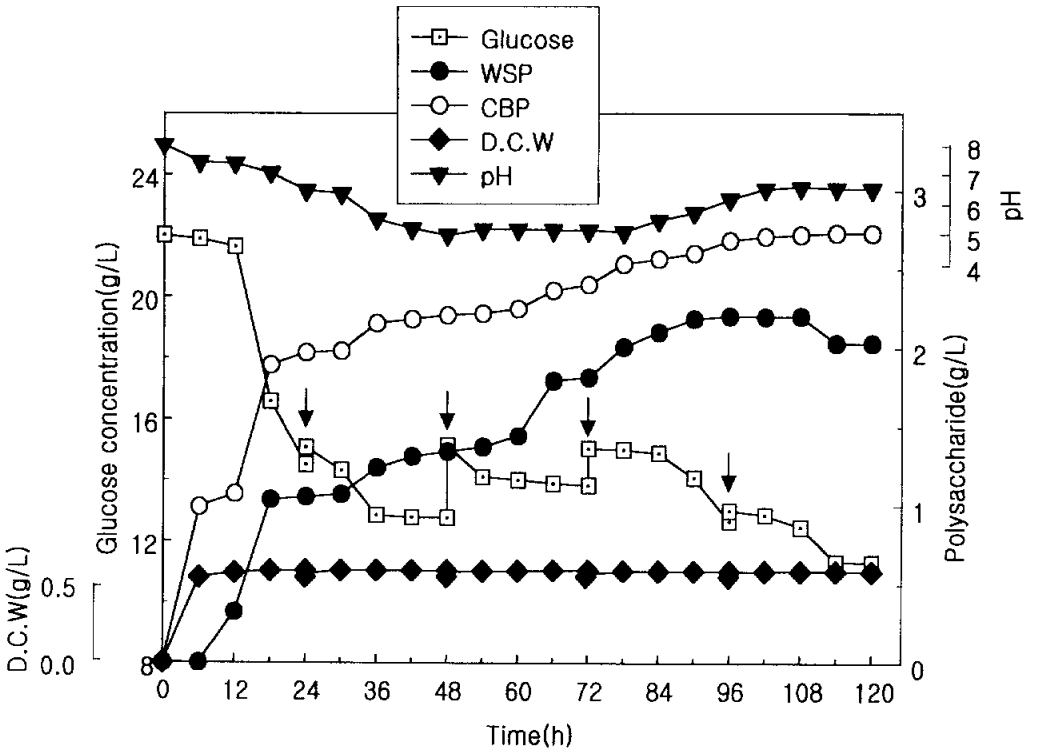


Fig. 12. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7L fermentor with the Fed-batch culture, 2.5 % Glucose. The arrows(↓) indicated the start of medium feeding each other (12.5 g glucose/500 mL medium).

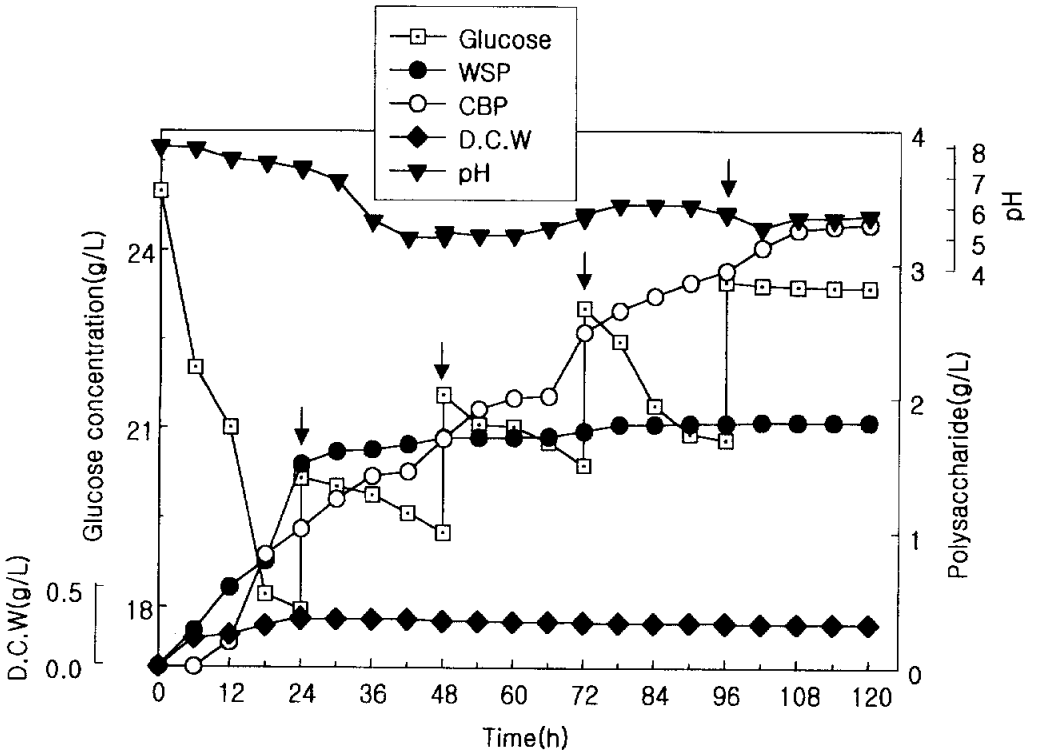


Fig. 13. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7 L fermentor with the Fed-batch culture, 2.5 % Glucose. The arrows(↓) indicated the start of medium feeding each other (10g glucose/200 mL medium).

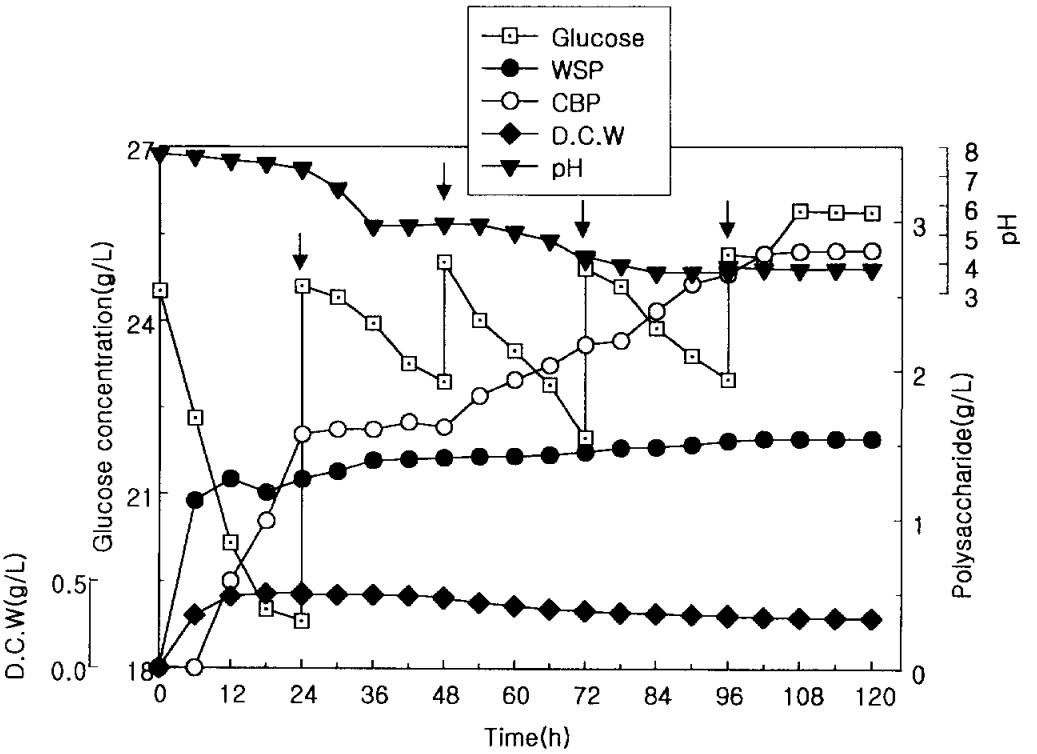


Fig. 14. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7 L fermentor with the Fed-batch culture, 2.5 % Glucose. The arrows(↓) indicated the start of medium feeding each other (20g glucose/200 mL medium).

4. Semi-continuous culture

The batch culture produced more the polysaccharides than the fed-batch culture. Because it is worth, as for this, feeding is unsuitable for fermentation conditions. Also, we carried out experimentation with the semi-continuous culture with a way to solve the high viscosity problem of the polysaccharides production from *Zoogloea* sp.[37].

The semi-continuous culture is the processes that can continuously keep cell growth with replaces a part of culture broth with new medium when growth of cells passes though exponential growth phase, and stop equipment worked in linear growth phase.

The most important experimentation factor is dilution ratio in the semi continuous culture and a dilution ratio is as follows[34,38].

$$D = \frac{V_n}{V_R}$$

D = Dilution ratio, V_n = New medium volume

V_R = Working volume

The experimentation carried out feeding volume(mL) of 200, 500 and 1000. In other words, dilution ratio is 0.04, 0.1 and 0.2, inoculum size (%, v/v) of 10 and 20. At the feeding time interval(h) were 12 and 24.

The results was turned out with the 500 mL feeding volume, 10 inoculum size (%, v/v), 24 feeding time interval(h) that the semi-continuous culture of optimal condition. When it was condition as this, production of the polysaccharides was 8.23 g/L after 144 h of cultivation [Fig.15] and the final volume of culture broth was 8 L.

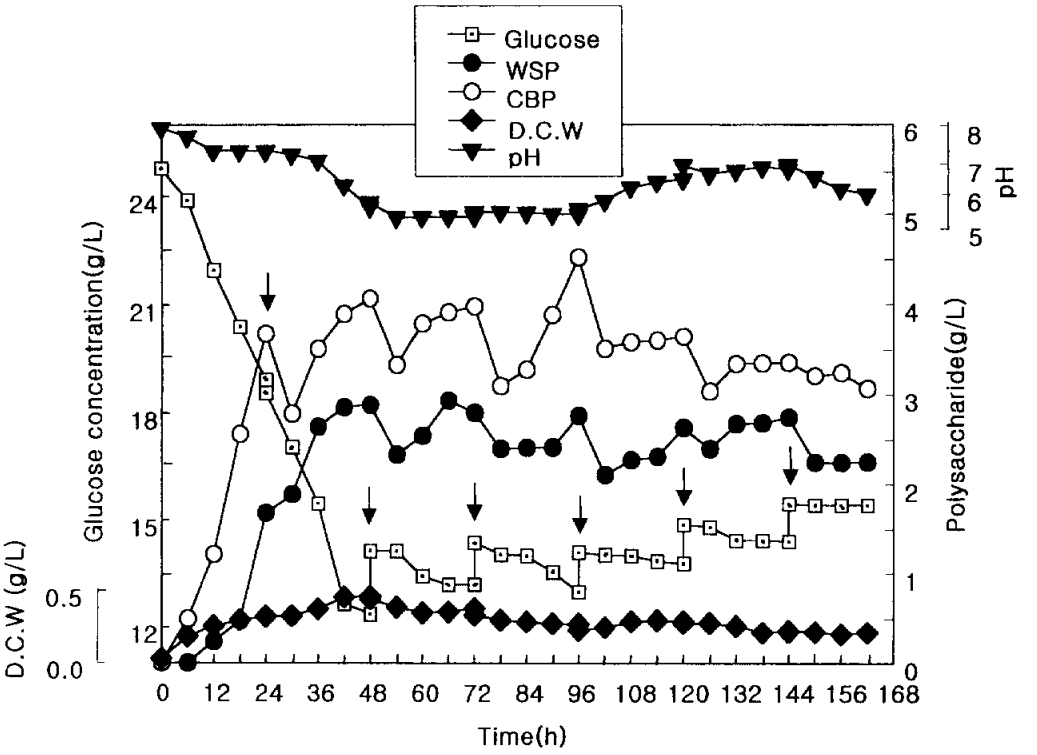


Fig. 15. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7 L fermentor with the Semi-continuous culture. The arrows(↓) indicated the time in which the medium (12.5 g glucose/500 mL medium) has been replaced.

4-1. Feeding volume

In this study, we examined the polysaccharides production by patterns [Table 4] of feeding volume. Feeding volume of 200 mL was hard to overcome high viscosity, and hyper-production was hard with excessive volume in case of 1000 mL[Fig.16,17][43].

The polysaccharides production in feeding volume were 6.59, 8.23 and 4.17 g/L, respectively. Therefore, the optimal condition was 500 mL[Fig.15].

4-2. Inoculum size

For comparison, examined an effect of the polysaccharides production with inoculum size(%, v/v) of 10 and 20[Table 4].

The polysaccharides production were 8.23 and 5.25 g/L, respectively. An injection held 20 % inoculum in order to reduce the cells loss which occurred that feed has been replaced. Therefore, production of the polysaccharides decreased to growth inhibition of cell or death and cells were used as an energy for a breathing with rather a lot of cells[Fig.18]. Consequently, the optimal condition of inoculum size was 10 %(v/v)[Fig.16][44,45].

4-3. Feeding time

Experimentation carried out conditions of feeding time interval executed 12 and 24 h with a solution way to overcome a problem of high viscosity.

According to the experimentation results, probably determine that an interval for 12 h influenced of the polysaccharides production and cells by excessive dilution[Fig.19][46].

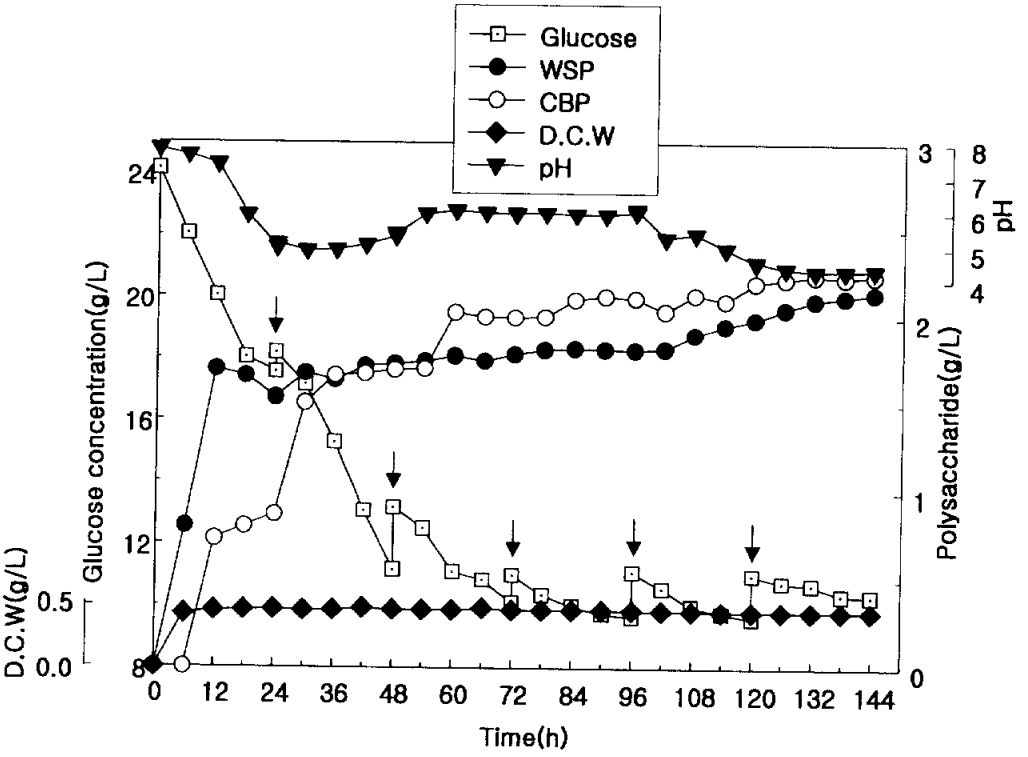


Fig. 16. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7 L fermentor with the Semi-continuous culture. The arrows(↓) indicated the time in which the medium (5 g glucose/200 mL medium) has been replaced.

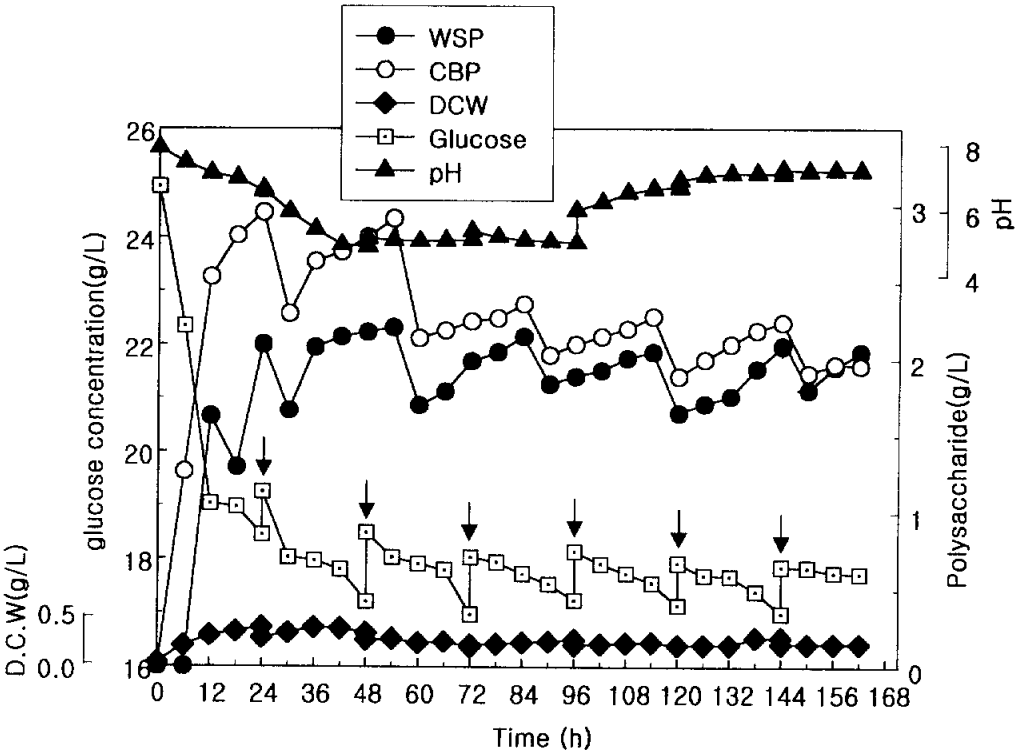


Fig. 17. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7 L fermentor with the Semi-continuous culture. The arrows(↓) indicated the time in which the medium (25 g glucose/1000 mL medium) has been replaced. Supply with feed in a interval for 24h.

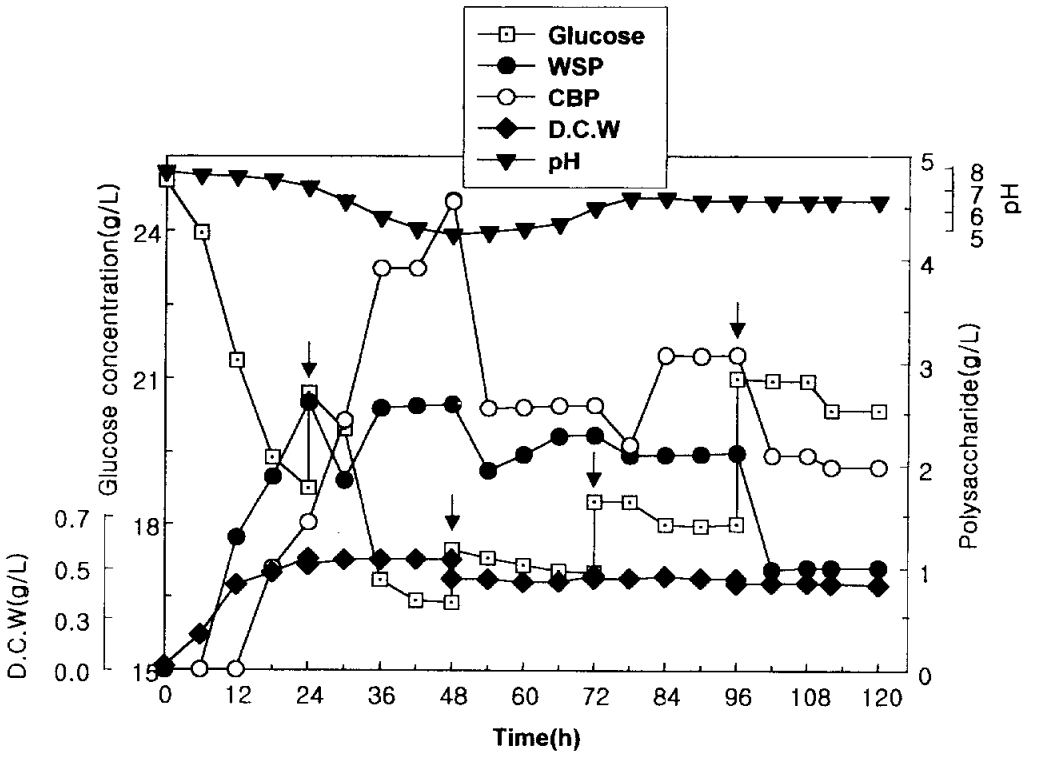


Fig. 18. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7 L fermentor with the Semi-continuous culture. 20 % inoculum. The arrows(↓) indicated the time in which the medium(12.5 g glucose/500 mL medium) has been replaced.

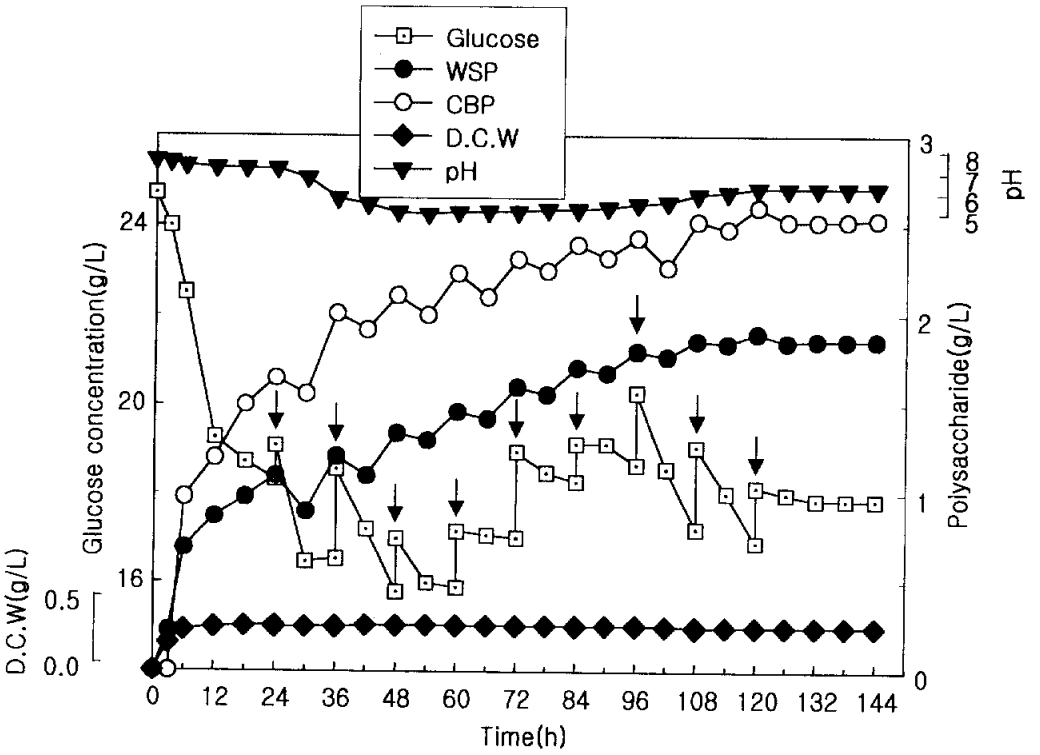


Fig. 19. Time course on the polysaccharides produced by marine bacterium *Zoogloea* sp. in 7 L fermentor with the Semi-continuous culture. The arrows(↓) indicated the time in which the medium (12.5 g glucose/500 mL medium) has been replaced. Supply with feed in an interval for 12h.

Table 4. Effect of various feeding conditions for the polysacccharides production in the semi-continuous culture

Feeding conditions		Cell concentration (g-D.C.W./L)	Polysaccharides Production(g/L)
Feeding volume (mL)	200	0.45	6.59
	500	0.50	8.23
	1000	0.36	4.17
Inoculum size (%, v/v)	10	0.50	8.23
	20	0.66	5.25
Feeding time interval(h)	12	0.35	5.91
	24	0.50	8.23

The polysaccharides production in feeding time were 5.91 and 8.23 g/L, respectively. Therefore, an interval was better the polysaccharides production on 24 h than an interval for 12h[Table 4][Fig.15].

5. Kinetics of fermentation

The logistic or modified logistic equations were employed to describe the kinetics of polysaccharides production[47-51].

We carried out the batch, fed-batch, semi-continuous culture for the polysaccharides production and its yield were 11.3, 6.28 and 8.23 g/L, respectively. However, productivity of the last polysaccharides of the batch, fed-batch and semi-continuous culture were 6.80, 4.77 and 9.75 g/L, respectively[Table 5].

Table 5. Comparison of the batch, fed-batch and semi-continuous culture for the polysaccharides production by *Zoogloea* sp.

Kinetic parameters	Batch culture	Fed batch culture	Semi continuous culture
Polysaccharides concentration (g/L)	11.34	6.276	8.226
Polysaccharides yield (g product / g glucose utilized)	0.995	0.510	0.777
Specific polysaccharides production rate (g polysaccharides / g biomass dry weight per day)	4.312	2.929	4.674
Biomass concentration (g/L)	0.526	0.430	0.309
Biomass yield (g biomass dry weight / g glucose utilized)	0.046	0.035	0.033
Specific biomass production rate (g biomass dry weight / g glucose utilized per day)	0.009	0.007	0.007
Specific glucose uptake rate (g glucose / g biomass dry weight per day)	4.311	5.720	6.067
Polysaccharides final volume (g polysaccharides total volume / g day)	6.804	4.770	9.750

Ⅵ. CONCLUSIONS

A newly isolated marine bacterium, identified as *Zoogloea* sp.(KCCM10036), produced two fundamentally different extracellular polysaccharides : Water-Soluble Polysaccharide (WSP) and Cell-Bound Polysaccharide(CBP). These polysaccharides, WSP and CBP showed anti-tumor activity and the useful characteristics of biopolymer as an effective adsorbent of heavy metal ions and as a new matrix for enzyme immobilization. Especially, these polysaccharides showed a high viscosity and so its various applications for the industrial use are expected. Therefore, the polysaccharides were produced by the batch, fed-batch and semi-continuous culture condition. The optimum nitrogen source and C/N ratio were bacto peptone and 5.

The optimum of the batch cultures were a 3L working volume, 400 rpm, 1vvm, 30 ℃, 2.5 %(w/v) glucose as a carbon source, 10 % (v/v) inoculum size. These polysaccharides production was 11.3 g/ℓ after 120 h of cultivation.

The optimal conditions of the fed-batch culture, feeding volume and concentration of feeding substrate were 200 mL and 2.5 %(v/v) glucose. At the feeding medium was supplied in an interval for 24 h and the polysaccharides production was 6.28 g/L after 120 h of cultivation and the final volume of culture broth was 3.8 L.

The semi-continuous culture of optimal conditions turned out with the 500 mL feeding volume, 10 %(v/v) inoculum size. At the feeding time interval was 24 h. When it was condition as this, production of the polysaccharides was 8.23 g/L after 144 h of cultivation and the final volume of culture broth was 8 L.

We carried out the batch, fed-batch, semi-continuous culture for the polysaccharides production and its yield were 11.3, 6.28 and 8.23 g/L, respectively. However, productivity of the last polysaccharides of the batch, fed-batch and semi-continuous culture were 6.80, 4.77 and 9.75 g/L, respectively.

Consequently, the optimal condition of the semi-continuous culture were identified with the 500 mL feeding volume, 10 %(v/v) inoculum size. At the feeding time interval was 24 h. Therefore, the semi-continuous culture proved to be the most effective fermentation mode for the polysaccharides production from *Zoogloea* sp.(KCCM10036).

V. 국문 초록

해양으로부터 분리된 *Zoogloea* sp.(KCCM 10036)는 Water-Soluble Polysa-ccharide (WSP)와 Cell-Bound Polysaccharide (CBP)의 두가지 서로 다른 고점성 다당류를 생산하는 특성을 지니고 있다. 또한 이러한 다당류는 항암효과 및 중금속 흡착능, 고정화지지체 등이 밝혀짐에 따라 산업적 응용가능성을 제시한 바 있다. 이러한 유용한 생물소재인 *Zoogloea* sp.로부터 고점성의 polysaccharides를 효과적인 방법으로 생산하기 위해 회분식, 유가식, 반연속식 배양을 수행하였다. 최적 질소원은 Bacto peptone이며, C/N ratio는 5 였다. 회분식 배양은 7 L 발효조에서 3 L working volume, 400 rpm, 1 vvm, 30 ℃, 2.5 %(w/v) glucose, 균접종량 10 %(v/v), 이와 같은 조건으로 120시간 배양시 11.3 g/L polysaccharides를 생산할 수 있었다. 유가식 배양은 2.5 %(w/v) glucose를 200 mL씩 24시간 간격으로 공급하여 120시간 동안 배양하였을 때, 6.28 g/L의 polysaccharides가 생산되었으며, 최종생산량은 3.8 L 였다. 그리고 반연속식 배양에서는 균접종량 10 %(v/v), 24시간 간격으로 500 mL의배지를 공급하여 144시간 동안 배양하여 8.23 g/L을 생산하였고 최종생산량은 8 L 였다.

회분식, 유가식, 반연속식을 통한 polysaccharies의 production은 각각 11.3, 6.28 그리고 8.23 g/L 였다. 그러나, 최종 polysaccharides의 productivity는 각각 6.80, 4.77 그리고 9.75 g/L 였다.

결론적으로, *Zoogloea* sp.(KCCM10036)로부터 polysaccahrides를 생산하기 위해 반연속식 배양법이 가장 효과적인 방법인 것으로 사료된다.

Ⅵ. ACKNOWLEDGMENT

먼저, 이 한편의 부족한 논문이 이뤄지기까지 여기까지 이끌어주시고 항상 참된 길로 지도해 주신 공재열 교수님께 깊은 감사를 드립니다.
또한 논문이 이루어질 수 있도록 끝까지 항상 관심을 가져준 김중균 교수님과 여수대학교 생물공학과 김종덕 교수님께 감사를 드립니다.
늘 함께 하지는 못했지만 저에게 가르침을 주신 여수대학교 냉동공학과 김민용 교수님, 학위과정동안 기초학문을 배양해주신 홍용기 교수님, 공인수 교수님, 김성구 교수님, 이형호 교수님, 박남규 교수님께 감사를 드립니다. 또한 항상 형님처럼 자상하게 대해주신 김학주 박사님, 미국에서 생활하게 될 김봉조 박사님 그리고 멀고도 먼 캐나다에서 열심히 연구하고 계신 큰누나 처럼 대해주셨던 하순득 박사님께 고마움을 전합니다. 제가 2년 동안 이곳 연구실에서 함께 하며, 사랑으로 보살펴주고 모든 일에 조언을 아끼지 않은 황선희 박사님과, 타향에서 생활하며 저에게 의지가 되어준 예비박사 임동중 선배님께 감사를 전합니다. 그리고 늦게 공부를 시작하였지만 항상 최선을 다하는 모습을 보여준 저의 동기이자 형인 강성일 동기님에게도 좋은 일만 있길 바랍니다. 또한 나에게 어려울 때 큰 힘이 되어준 전병진 후배님과 캐나다에서 열심히 어학공부를 하고있는 김영문 후배님께 고맙다는 말을 하고싶습니다. 그리고 처음 연구실생활에 적응할 수 있도록 도와준 정혜성 선배님과 일본에서 공부하고 있는 이경미 후배님께 응원과 감사를 보냅니다. 그리고, 어렵게 일본에서 생활하고 있는 나의 하나뿐인 형이면서 연구실의 선배인 장재혁 선배님께 고마움과 격려를 보냅니다. 또한 항상 애정으로 격려해 주신 외조모님과 일가 친지분들께도 깊은 감사를 드립니다. 힘들 때 저에게 찾아와 힘이 되어준 친구 재성, 승한, 용해, 5713 회원들에게 감사를 드립니다. 끝으로, 우리 형제를 여기까지 이끌며 끊임없는 사랑과 노력으로 헌신적으로 고생하신 나의 부모님께 눈물로 한편의 논문을 바칩니다.

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