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Carbohydrate Polymers 71 (2008) 468–475
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Optimization for biopolymer production by *Enterobacter cloacae* WD7

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Received 21 December 2006; accepted 21 June 2007
Available online 30 June 2007

Abstract

Factors affecting the production of exopolysaccharide (EPS) from *Enterobacter cloacae* WD7 cultivated in basal medium (pH 7.0) using 1% w/v glucose as a carbon source for 5 days at 30 °C were investigated. The EPS yield of 2.23 g L^{-1} was obtained after 3 days cultivation. Among the carbon sources tested, 3% sucrose was selected and gave an EPS yield of 2.72 g L^{-1}. This indicated that sucrose is a better carbon source than the glucose used previously. The addition of either inorganic nitrogen [$(NH_4)_2SO_4$, NH_4Cl and NH_4NO_3] or organic nitrogen (polypeptone) sources had no effect on the EPS yield. The optimum concentration of yeast extract was found to be 0.05% w/v. The optimum environmental conditions were: initial pH 7.0, temperature 30 °C, pH control at 7.0 and the aeration rate of 2.0 vvm which elevated the EPS yield to 4.80 g L^{-1} while further increase in the agitation speed (200 rpm) reduced the production of EPS. Comparison of the kinetic parameters of the shake-flask and batch fermentations revealed that batch fermenter culture gave about 2 times higher values of specific growth rate ($\mu = 0.15$ and 0.29 h^{-1}), product yields ($Y_{p/s} = 0.25$ and 0.52 g EPS g sucrose^{-1}) and maximum productivity ($R_m = 0.04$ and 0.07 g of EPS L^{-1} h^{-1}). Substitution of the analytical grade sucrose by commercial sucrose resulted in 15% lower EPS yield (i.e. from 4.90 to 4.18 g L^{-1}, respectively) but could lower the raw material cost by 98%, and therefore is a suitable component for the production medium. For continuous culture studies, the optimum dilution rate was 0.05 h^{-1} giving the maximum EPS concentration of 7.28 g L^{-1} which is 1.5 times higher than the batch culture and a total of 3.2 times higher than the beginning (2.23 g L^{-1}). The critical dilution rate (D_c) was calculated to be 0.49 h^{-1} with the maximum dilution rate (D_m) of 0.485 h^{-1}, $Y_{x/s}$ of 0.03 g cell/g sucrose and the R_m of 0.06 g EPS L^{-1} h^{-1}. Batch culture therefore gave a slightly higher productivity and would be the method of choice for scale-up studies.

Keywords: Optimization; Biopolymer; *Enterobacter cloacae*; Batch culture; Continuous culture

1. Introduction

Most of the extracellular polymers produced by bacteria are polysaccharides, although a few bacteria produce capsules made up of polypeptides of amino acid residues. The molecular weight and even the composition of these extracellular polysaccharides may vary depending on the culture conditions (Sutherland, 1990). Several microbial polysaccharides are now produced commercially from many species of bacteria, as well as from some marine algae, fungi and plants (Lee, 1996). The most important microbial polysaccharide is xanthan, the commercial polymer produced by the bacteria *Xanthomonas campestris*, but others include dextran, alginate and pullulan (Blanch & Clark, 1996). The exopolysaccharides produced by microorganisms are widely used in the food, pharmaceutical and chemical industries, and function as bioflocculants, bioabsorbents, heavy metal removal agents, drug delivery agents, etc (Bender, Eaton, Ekanemesang, & Phillips, 1994).

Microbial flocculants have been suggested to replace organic high-polymer flocculants, such as polyacrylamide, which are inexpensive and highly effective but not easily

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0144-8617/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2007.06.017

degraded and can be harmful to humans. Among the microbial flocculants reported previously, protein polysaccharide, glycoprotein, polyglutamic acid and xanthan have been investigated (Yokoi et al., 1997). In our previous studies (Prasertsan, Dermlim, Doelle, & Kennedy, 2006), the newly isolated *E. cloacae* WD7 was found to produce exopolysaccharide (EPS) possessing high flocculating activity with the EPS yield of 2.27 g L^{-1}. This paper describes the continuation of process development of exopolysaccharide production from *E. cloacae* WD7 with the emphasis on optimization of medium and environmental condition during the fermentation process in order to increase the EPS yield.

2. Materials and methods

2.1. Microorganism

Enterobacter cloacae WD7 was isolated from activated sludge of a seafood processing plant by Prasertsan et al. (2006).

2.2. Medium

Basal medium contained (g L^{-1}): glucose, 10, $(NH_4)_2SO_4$, 0.5, polypeptone, 2, yeast extract, 0.5, K_2HPO_4, 2, $MgSO_4{\cdot}7H_2O$, 0.5 and NaCl, 0.1 (Yokoi, Natsuda, Hirose, Hayashi, & Takasaka, 1995).

2.3. Analytical methods

Cell growth was measured by diluting the culture broth with distilled water to obtain optimum dilution. After mixing, the absorbance was measured at 660 nm (Kurane et al., 1994).

Dry cell weight was determined by centrifugation of diluted culture broth (50 ml) at 12,846*g* for 15 min at 5 °C. The cell sediments were washed by resuspending in distilled water (50 ml) and repeated centrifugation. The washed cells were dried for 24 h at 105 °C and then weighed to constant weight after cooling in a desiccator (Dermlim, Prasertsan, & Doelle, 1999).

Crude exopolysaccharide (EPS) yield was determined by diluting the supernatant with 4 volumes of cold 95% ethanol and leaving for 2 h at −20 °C for precipitation to be complete, and centrifuging at 7600*g* for 15 min at 5 °C. The precipitate was dried for 24 h at 105 °C and weighed to constant weight after cooling in a desiccator (adapted from Dermlim et al., 1999).

Residual sugar (sucrose) in the diluted supernatant was determined by HPLC using a Shim-pack CLC-NH_2 column and a refractive index detector. Acetonitrile/water (74:26) was used as the mobile phase at the flow rate of 1.5 ml min^{-1}. The standard sugar solutions of 0.05% and 1.0% sucrose were prepared and used as a control (Adapted from Liu & Steinbuchel, 1997).

2.4. Batch culture

2.4.1. Effect of nutrients and environmental condition on EPS production

One loopfull of a 24 h culture of *E. cloacae* WD7 was inoculated into basal medium (200 ml) in a 500 ml flask and cultivated on a rotary shaker (200 rpm) at 30 °C for 12 h. The culture was diluted with sterile distilled water to obtain viable cell counts of 10^8 CFU ml^{-1}. This starter culture (5% v/v) was then added into 200 ml fresh basal medium. Cultivation was performed on a rotary shaker at 30 °C for 3 days.

The influence of different carbon sources (glucose, fructose, galactose, sucrose and maltose at 1% w/v) and concentrations (0–4.0% w/v) of the selected carbon sources were studied. The effects of inorganic nitrogen sources [$NH_{42}SO_4$, NH_4NO_3 and NH_4Cl] at 0.05–0.15% w/v and organic nitrogen (polypeptone) at 0–0.3% w/v were tested. Yeast extract concentrations were varied from 0% to 0.20% w/v. Studies on the effect of initial pH were made in the range 6.0–8.0, and the range for incubation temperature was made within 30–40 °C. Cultivation under controlled pH (at the optimal initial pH value) were conducted in a 3-L fermenter containing optimal medium (1.8 L) at an aeration rate of 0.5 vvm and a 200 rpm agitation rate. The other environmental parameters investigated were the aeration rates (0, 0.5, 1.0 and 2.0 vvm), and agitation speed (200, 400, 600 and 800 rpm). Samples were taken to measure for pH, dry cell weight, crude EPS yield and residual sugar.

The following kinetics parameters were determined ; the specific growth rate (μ), maximum specific growth rate (μ_m), cellular yield coefficient ($Y_{x/s}$), conversion yield of substrate to product ($Y_{p/s}$), specific rate of product formation (q_p), specific rate of substrate utilization (q_s), maximum productivity (R_m), generation time (g) and saturation constant (K_s) (Pirt, 1975). The K_s value was determined by cultivation in the fermenter with various concentrations of sucrose (0–4%).

2.4.2. Effect of commercial sucrose on EPS production

Modification of the optimum synthetic medium was investigated by replacing the analytical grade sucrose with commercial white sugar sucrose obtained from the supermarket. The effect of sucrose concentrations were tested at 1% and 3% (w/v).

2.5. Continuous culture

2.5.1. Effect of dilution rate

The batch cultivation was carried out in a 3 L fermenter containing 1.8 L of the optimal medium under the optimal environmental condition for 3 days, then starting to feed the optimal medium at different dilution rates (D) at 0.01, 0.05 and 0.10 h^{-1}. The dilution rate was calculated based on the equation

$$\text{Dilution rate } (D), \text{h}^{-1} = \frac{\text{Medium flow rate } (F), \text{L h}^{-1}}{\text{Culture volume } (V), \text{L}}$$

Kinetic parameters for continuous culture were calculated for critical dilution rate (D_c), maximum dilution rate (D_m), cellular yield coefficient ($Y_{x/s}$), and maximum productivity (R_m) (Pirt, 1975).

3. Results and discussion

3.1. Batch culture

3.1.1. Effect of nutrients and environmental conditions on EPS production

The exopolysaccharide (EPS) produced from *E. cloacae* WD7 are acidic heteropolysaccharides consisting of neutral sugar (29.4%) and uronic acids (14.18%) (Prasertsan et al., 2006). Time course studies were conducted on growth and EPS production by *E. cloacae* WD7 in basal medium (pH 7.0) using 1% glucose as carbon source for 5 days at 30 °C (Fig. 1). The bacterium grew rapidly within the first 6 h, which was correlated with the rapid decline of pH, which was due to the sugar being metabolized by cells and the cells forming acidic metabolites and EPS (Lawson & Sutherland, 1978; Clarke, Bailey, Roberts, & Tsang, 1991). After 6 h, the pH increased rapidly which may have been caused by the generation of ammonia from nitrogen source consumption and became constant after 24 h. The highest EPS yield (2.23 g L^{-1}) was obtained after 3 days cultivation which was in the stationary phase.

The culture broth appeared viscous as expected since the EPS from *E. cloacae* WD7 was previously reported to contain hydroxyl groups enabling it to become hydrated, swell and yield viscous solutions (Prasertsan et al., 2006). It has been suggested that the bacterial polymer could be biosynthesized through either a block mechanism or a monomeric mechanism (Shibaev, 1986). *Enterobacter cloacae* WD7 is anticipated to synthesize the EPS via the second mechanism as do almost all of the Enterobacteriaceae (Tonn & Gander, 1979). After it has been synthesized, the polysaccharide would be released into the culture broth (Sutherland & Ellwood, 1979).

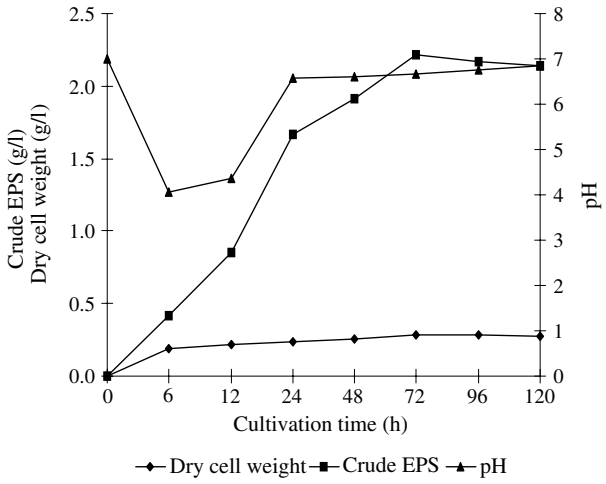

Fig. 1. Time course on EPS production and growth of *E. cloacae* WD7 in basal medium under shake-flask culture conditions.

The effects of each medium component and environmental conditions on cell growth and EPS formation of *E. cloacae* WD7 are summarized in Table 1 and details are as follows.

Effect of carbon source and concentration. Cell growth and EPS production usually depends on carbon sources which affect quality, sugar component and/or molecular weight of EPS (Wachenheim & Patterson, 1992). The data for effects of carbon sources (1%) on EPS production from *E. cloacae* WD7 showed that sucrose and galactose gave similar EPS yields (2.45 and 2.50 g L^{-1}, respectively) ($p < 0.05$) and higher than those from using maltose, fructose and glucose (2.34, 2.32 and 2.23 g L^{-1}, respectively). Sucrose was chosen due to its lower cost than galactose. Increase of the sucrose concentration (testing at 1%, 2%, 3%, 4% w/v) resulted in increase in EPS yields of 2.23, 2.24, 2.72, and 2.52 g L^{-1}, respectively. The optimal sucrose concentration for EPS production from *E. cloacae* WD7 was 3% w/v while it was 2.5% w/v for *Agrobacterium tumefaciens* (Stredansky & Conti, 1999), 10% for *Lactobacillus* strain LB 80 (Geel-Schutten, Flesch, Brink, Smith, & Dijkhuizen, 1998) and 2% w/v for *Lactobacillus casei* CG11 (Cerning et al., 1994), giving the maximum EPS yields of 2.72, 23.5, 21.0 and 0.05 g L^{-1}, respectively.

Effect of nitrogen source and concentration. Addition of either inorganic nitrogen sources [NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$] or organic nitrogen (polypeptone) was found to influence growth of *E. cloacae* WD7 with the highest value in the presence of 0.05% NH_4Cl (OD_{660} of 3.78) and 0.3% polypeptone (OD_{660} of 2.67), respectively. However, addition of inorganic nitrogen into the basal medium had no effect on EPS formation from *E. cloacae* WD7 as there was enough nitrogen available from organic nitrogen (0.2% polypeptone and 0.05% yeast extract) in the basal medium. Too high nitrogen concentration in the medium also resulted in poor xanthan production (Lo, Yang, & Min, 1997). Addition of inorganic nitrogen (0.2% NH_4NO_3) (Lawson & Sutherland, 1978), urea and potassium nitrate could increase the production of exopolysaccharide (Zanflo) from *Erwinia herbicola*, and the production of bioabsorbent by *Alcaligenes latus* B-16 (Nohata & Kurane, 1994), respectively. The high C:N ratio (60 to 1) of basal medium in the current work gave higher EPS yield with lower cell growth because the pathway for cell growth was blocked and changed to the EPS biosynthesis pathway. Nevertheless, nitrogen source should be available at the beginning of the stationary phase (Lo et al., 1997). Xanthan production was poor if the nitrogen concentration in the medium was too high (Lo et al., 1997).

Effect of yeast extract concentration. The EPS yield from *E. cloacae* WD7 decreased as yeast extract concentrations increased in the range of 0–0.2%. Cell growth, however, increased as the concentrations of yeast extract increased

Table 1
The optimal conditions for a high EPS yield production from *E. cloacae* WD7 cultivated in shake-flasks and 3 L batch fermenter

Optimal condition	EPS yield (g L^{-1})
Shake-flask culture	
Carbon sources (1%)	
Glucose	2.23
Sucrose	2.45
Galactose	2.50
Maltose	2.34
Fructose	2.32
Sucrose concentrations (%)	
0	0.09
1	2.23
2	2.24
3	2.72
4	2.52
Inorganic nitrogen sources (0.05%)	
No addition	2.65
$(NH_4)_2SO_4$	2.51
NH_4NO_3	2.53
NH_4Cl	1.69
Organic nitrogen (polypeptone) concentrations (%)	
0	2.70
0.1	2.01
0.2	2.00
0.3	1.85
Yeast extract concentrations (%)	
0	1.97
0.05	2.71
0.1	2.68
0.2	1.75
Initial pH	
6	1.32
6.5	1.97
7	2.72
7.5	2.36
8	2.07
Incubation temperature (°C)	
30	2.71
35	1.20
40	0.66
3 L batch fermenter culture	
Control of pH	2.91
Controlled	2.11
Uncontrolled	
Aeration rates (vvm)	
0	1.26
0.5	2.96
1.0	3.79
2.0	4.80
Agitation speed (rpm)	
200	4.82
400	4.78
600	4.50
800	4.30

($p < 0.05$). The highest EPS yield (2.71 g L^{-1}) was achieved at 0.05% yeast extract compared to 1.97 g L^{-1} without any yeast extract added. Increasing yeast extract concentrations as sole nitrogen source also gave higher pullulan yields (11.0 g L^{-1} EPS) from a mixed culture of *Aureobasidium pullulans* and *Kluyveromyces fragilis* (Shin, Kim, Lee, Cho, & Byun, 1989). One or more component(s) of yeast extract not only affected the polydispersity of zooglan produced by *Zoogloea ramigera* 115SLR but also the activities of polymerase or depolymerase or enzyme synthesis (Guillouet, Choi, Rha, & Sinskey, 1999).

Effect of initial pH. Unlike other initial parameters, the initial pH of 7.0 was found to be optimum for both cell growth and EPS production by *E. cloacae* WD7. It was reported that pH had more influence on polysaccharide production than on cell growth (Pace, 1981) and the specific pH affected directly the synthesis of those enzymes responsible for EPS production (Lawson & Sutherland, 1978). This optimum pH for growth and EPS production from *E. cloacae* WD7 was at 7.0 while it was pH 7.2 from *E. herbicola* (Keith et al., 1991). This was within the optimum pH range (6.0–7.5) for the synthesis of polysaccharides (Lawson & Sutherland, 1978). The highest EPS yield obtained in the current work was 2.7 g L^{-1}. The initial pH also affected the molecular weight of the product (Jeanes, 1977), for example, the initial pH of 5–7 and 7–8 gave high ($1.5–4 \times 10^6$) and low ($5–10 \times 10^4$) molecular weight pullulan, respectively, from *A. pullulans* (Margaritis & Pace, 1985). For xanthan production by *X. campestris*, the pH must not be lower than 5.0. Acidic conditions give lower yields because the polymer could be hydrolyzed at low pH (Clarke et al., 1991).

Effect of temperature. Incubation temperature is often a critical factor in polysaccharide biosynthesis. All commercial polysaccharide-producing microorganisms are mesophiles (Lawson & Sutherland, 1978). The optimum temperature for polysaccharide production depends on the type of microorganism. The optimum temperature for EPS production from *E. cloacae* WD7 was 30 °C which was similar to those of other bacterial strains such as 30 °C for *E. herbicola*, 25–29 °C for *Alteromonas* HYD-1545 and 28 °C for *X. campestris* (Vincent et al., 1994), and 30 °C for *X. campestris* with no growth occurring at 35 °C (Shu & Yang, 1991). The optimum temperature for polysaccharide production by lactic acid producing bacteria must be lower than growth temperature. The association of low cell mass with greater polysaccharide production has been explained by Sutherland (1990) and in that work when cells grew slowly, synthesis of cell wall polymer was also slower, making more isoprenoid phosphate available for exopolymer synthesis. In the present work it was noted that the EPS yield of *E. cloacae* WD7 reduced by 50% for every 5 °C increase of temperature.

Effect of pH control. In cultivation in a 3 L fermenter to control pH at 7.0, *E. cloacae* WD7 was able to grow well both under uncontrolled and controlled pH (7.0) during the first 2 days of cultivation and growth continued to increase in the case of controlled pH. As a consequence, the higher crude EPS yield (2.91 g L^{-1}) was achieved under pH control condition than that of uncontrolled pH

condition (2.11 g L^{-1}). This influence of pH control on the increase of EPS yield was observed by Dlamini and Peiris (1997) and (Gassem, Schmidt, & Frank, 1997) as greater viscosity was achieved under controlled pH condition. Control of pH is also needed as the polysaccharides of commercial interest are generally acidic (the acidic properties of the biopolymers caused decrease of pH, hence more alkali is needed to be added to maintain the normal pH) (Pace, 1981) including the EPS from *E. cloacae* WD7 (Prasertsan et al., 2006).

Effect of aeration rate. The rate of dissolved oxygen supply must at least equal the rate of oxygen demand (Gerhardt & Drew, 1994). Aeration plays a major role in the polymer production since the oxygen transfer becomes a limiting factor as the polymer concentration, as well as viscosity, increase. In this study, aeration rate had a profound effect on the crude EPS yield of *E. cloacae* WD7 as it significantly increased both EPS production and cell growth. Besides the EPS yield, aeration might indirectly affect the overall production rate (Margaritis & Pace, 1985). The higher EPS yields in the present work were correlated with cell growth and dissolved oxygen consumption, although only a slight increase of cell growth occurred at higher aeration rate. The highest aeration rate tested at 2.0 vvm gave the maximum EPS yield of 4.8 g L^{-1}, which is 3.8-fold increase above that from unaerated conditions (1.26 g L^{-1}) and a 1.6-fold increase from the highest value obtained from the study on the effect of pH control (2.91 g L^{-1}).

Effect of agitation speed. The EPS yield and growth decreased as the agitation speed increased from 200 to 800 rpm. This may be due to the fact that oxygen supply at 2.0 vvm was adequate and further increase through agitation was not necessary. The agitation rate of 200 rpm was therefore selected. The decline of cell growth at higher agitation speed may be due to cell destruction. Dissolved oxygen curves showed that higher agitation speeds gave higher dissolved oxygen levels ($p < 0.05$) with the dissolved oxygen value over 60%, 70%, 80% and 90% of total dissolved oxygen at 200, 400, 600 and 800 rpm, respectively. The oxygen availability by controlled aeration rate and agitation speed can maintain the dissolved oxygen at not less than 30% (in this case 60%) which is suitable for polymer production (McNeely, 1967). High agitation rates (750 rpm and above) clearly resulted in substantially diminished exopolysaccharide yields by *A. pullulans* compared to those at lower speeds (125–500 rpm) while biomass yield increased (Gibbs & Seviour, 1996).

For xanthan production, the critical oxygen level was found to be between 6% and 10%, below 6% both specific xanthan production rate as well as specific oxygen uptake rate decreased significantly (Amanullah, Tuttiett, & Nienow, 1998). However, alginate production by *Azotobacter vinelandii* was enhanced by the microaerobic conditions (3–5% pO_2) (Sabra, Zeng, Sabry, Omar, & Deckwer, 1999). Therefore, the optimum concentration of oxygen depends on the strain of microorganism.

Results on the cultivation of *E. cloacae* WD7 in the fermenter using the optimal medium and environmental condition (pH controlled at 7.0 and incubation at 30 °C for 3 days with aeration rate of 2.0 vvm and agitation speed of 200 rpm) is given in Fig. 2. *Enterobacter cloacae* WD7 grew rapidly from the beginning due to the inoculation of the starter culture. The EPS started to produce after 12 h cultivation and increased sharply till 36 h, then increased slightly till the end of fermentation. The kinetic parameters of batch cultivation in shake-flask and fermenter are given in Table 2. The kinetics values obtained from cultivation in the fermenter were about 2 times higher than those from batch shake-flask culture for: the specific growth rate ($\mu = 0.15$ and 0.29 h^{-1}), the maximum specific growth rate ($\mu_m = 0.25$ and 0.49 h^{-1}), the generation time (2.39 and 4.62 h), the product yields ($Y_{p/s} = 0.25$ and 0.52 g EPS g sucrose^{-1}), the productivity ($R_m = 0.04$ and 0.07 g EPS L^{-1} h^{-1}) and the saturation constant ($K_s = 1.30 \times 10^{-5}$ and 2.60×10^{-5} g sucrose L^{-1}). In addition, the specific rates of product formation ($q_p = 0.60$ and 0.56 h^{-1}) were similar while the specific rate of substrate utilization ($q_s = 5.0$ and 7.25 h^{-1}) of continuous culture was 1.5 times higher than the shake-flask culture.

The specific growth rate of *E. cloacae* WD7 (0.29 h^{-1}) was similar to that of *Methylobacterium organophilum* for production of methylan (0.23 h^{-1}) (Oh, Kim, & Yoshida, 1997) but 3 times higher than that of pullulan production from *A. pullulan* (0.09 h^{-1}) (Boa & LeDuy, 1987). Conversion of 70–80% of utilized glucose into crude polymer is commonly found in high yielding polysaccharide fermentations (Margaritis & Pace, 1985). *E. cloacae* WD7 had quite high conversion yield of substrate to product (0.52 g EPS g sucrose^{-1} or 52%) when compared to that from *Alteromonas* HYD-1545 using glucose as a carbon source (37%) (Vincent et al., 1994).

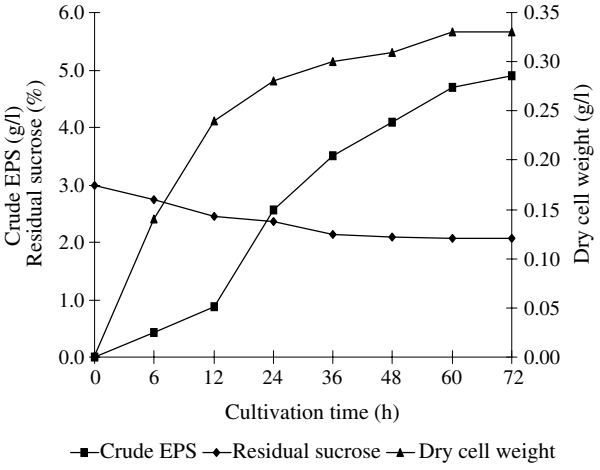

Fig. 2. Growth and exopolysaccharide (EPS) production from *E. cloacae* WD7 cultivation in a 3-l fermenter at 30 °C, with aeration rate of 2.0 vvm, agitation speed of 200 rpm and pH control at 7.0.

Table 2
Kinetic parameters and EPS production from *Enterobacter cloacae* WD7 under optimal medium and conditions of shake-flask and batch fermenter

Kinetic parameters	Shake-flask	Batch fermenter
Specific growth rate (μ), h^{-1}	0.15	0.29
Maximum specific growth rate (μ_m), h^{-1}	0.25	0.49
Cellular yield coefficient ($Y_{x/s}$), g cell/g sucrose	0.03	0.04
Conversion yield of substrate to product ($Y_{p/s}$), g crude EPS/g $sucrose^{-1}$	0.25	0.52
Specific rate of product formation (q_p), h^{-1}	0.60	0.56
Specific rate of substrate utilization (q_s), h^{-1}	5.0	7.25
Maximum productivity (R_m), g crude EPS L^{-1} h^{-1}	0.04	0.07
Generation time (g), h	4.62	2.39
Saturation constant (K_s), g sucrose L^{-1}	1.30×10^{-5}	2.60×10^{-5}
EPS conc. (g L^{-1})	2.71	4.80

3.1.2. Effect of commercial sucrose on EPS production

Substitution of analytical grade sucrose by commercial grade sucrose gave slightly lower EPS yield both at 1% and 3% sucrose concentrations (Fig. 3). Higher EPS yield from using analytical grade sucrose may have been due to its slightly higher purity than the commercial sucrose (Stredansky, Conti, Bertocchi, Matulova, & Zanetti, 1998). Some impurities in commercial sucrose may suppress or inhibit enzymes involved in polymer production. Nevertheless, the maximum yield of EPS from these two sources at 3% concentration was only a 15% difference, 4.18 and 4.90 g L^{-1}, respectively, while the price of the analytical grade sucrose was 98% higher. Hence, it is more economical to replace the analytical sucrose by commercial sucrose. It is well known that commercial carbon sources are always used for commercial scale productions because of its lower cost. Some reports revealed that commercial carbon sources such as molasses, fruit juice and various agro-industrial wastes gave higher polymer yields than those from analytical sources (Clarke et al., 1991; Lawson & Sutherland, 1978; Roukas, 1998). However, these agro-industrial wastes should be supplemented with other components such as nitrogen and phosphorus sources in order to obtain the maximum yield, as shown in the case of pullulan (30.8 g L^{-1}) – indicating the very large amount of pullulan produced and supporting the fact that despite any impurity, high yield could be achieved from agro-industrial wastes (Israilides et al., 1998).

3.2. Continuous culture

3.2.1. Effect of dilution rate

The continuous culture was performed in a 3 L fermenter with 1.8 L working volume of the optimal medium for EPS production containing 3% sucrose, 0.05% yeast extract, 0.2% KH_2PO_4, 0.05% $MgSO_4 \cdot 7\,H_2O$ and 0.01% NaCl with the initial pH of 7.0 at 30 °C. Cultivation was conducted under the optimum condition with the control of pH at 7.0, aeration rate of 2.0 vvm and the agitation speed of 200 rpm. The optimum dilution rate was found to be 0.05 h^{-1} which gave the maximum of both cell growth ($OD_{660} = 1.24$) and EPS production (7.28 g L^{-1}) (Fig. 4). This optimum dilution rate was lower than those of 0.15 h^{-1} for alginate production from *A. vinelandii* (Sabra et al., 1999) and 0.075 h^{-1} for pullulan production from *A. pullulans* (McNeil, Kristiansen, & Seviour, 1989).

Based on the kinetics parameters of batch culture, the kinetics of the continuous culture at the optimum dilution

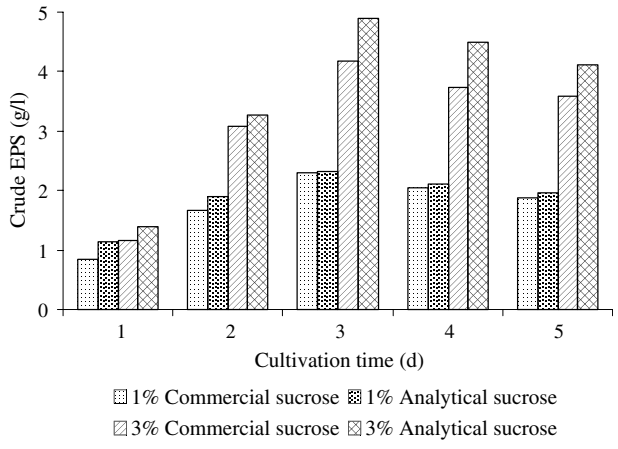

Fig. 3. The exopolysaccharide (EPS) production from *E. cloacae* WD7 cultivated in optimal medium containing commercial and analytical sucrose under optimal condition.

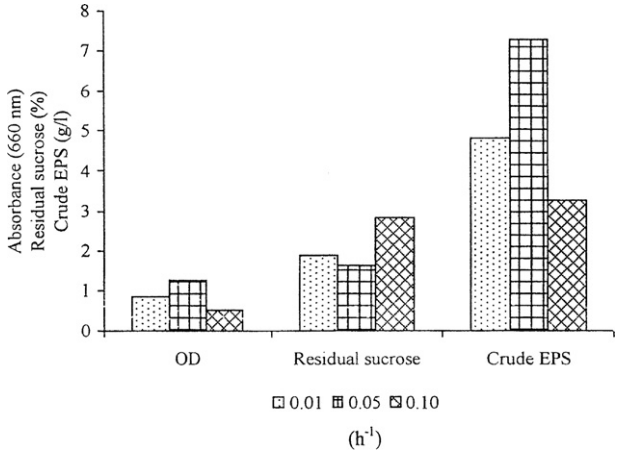

Fig. 4. Effect of dilution rate on growth, residual sucrose and exopolysaccharide production from *Enterobacter cloacae* WD7 in continuous cultivation feeding with the optimal medium.

rate of 0.05 h^{-1} are calculated. The critical dilution rate was 0.49 h^{-1}, the maximum dilution rate was 0.485, the cellular yield coefficient ($Y_{x/s}$) was 0.03 g cell/g sucrose and the maximum productivity (R_m) was 0.06 g crude EPS L^{-1} h^{-1}. This critical dilution rate (0.49 h^{-1}) was 4 times higher than that of *Acetobacter xylinum* subsp. *Sucrofermentans* (0.12 h^{-1}) for cellulose production (Naritomi, Kuoda, Yano, & Yoshinaga, 1998).

4. Conclusions

Optimization of medium and environmental conditions for process development of exopolysaccharide possessing flocculating activities from *E. cloacae* WD7 showed that the optimum medium contained 3% w/v sucrose, 0.05% w/v yeast extract, no addition of any nitrogen source in the basal medium with the initial pH of 7.0. The optimum condition during cultivation was controlling pH at 7.0 at 30 °C with the aeration rate of 2.0 vvm. Batch cultivation gave a slightly higher polymer productivity than the continuous culture. These lab-scale results provide essential information needed for this process development on scaling up study in a 72 L fermenter. This EPS industrial scale production and application are potentially important given the results of investigation of the properties of this EPS.

Acknowledgement

The authors thank Prince of Songkla University for the Graduate Study for Outstanding Scholastic Achievement Scholarship to Mr. W. Santad and Graduate School for providing the research budget.

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