Growth characteristic of *Rhodococcus opacus* PD630 on MSM and glycerol

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Abstract

Rhodococcus opacus PD630, an oleaginous bacterium, was cultivated in the batch culture using MSM and 10%w/v glycerol as carbon source. Nitrogen source in MSM was changed from ammonium chloride to ammonium acetate. This work demonstrated that *R. opacus* PD630 consumed two carbon sources; glycerol and ammonium acetate; and grew obviously in two periods of time. First, it consumed acetate from nitrogen source, biomass increased rapidly from 0.068 g/L to 1.883 g/L after 2 days of the cultivation. Then, it started to consume glycerol, biomass increased rapidly again from 1.856 g/L to 2.353 g/L after 14 days of the cultivation. In this study, *R. opacus* PD630 could consume glycerol, but used long time, which showed the low efficiency to make the cell growth.

Keywords: *Rhodococcus opacus* PD630, glycerol, biomass

Introduction

Fuels are playing a major role in economy of most countries. The main energies needs in the world are supplied by the petroleum resources for transportation, industry and in many needs of human (Borugadda, 2012). The rising of petroleum fuels price, quantity depletion and the concern about environmental problems from the emission of pollutant gases force people to find the new sources of energy. Biodiesel is an alternative and renewable fuel. Transesterification of vegetable oils (triglycerides) with methanol is a process that produces biodiesel (fatty acid methyl esters) and glycerol as co-product. Every 100 kg of biodiesel produced, 10 kg of glycerol also produced (Ayoub and Ahmad, 2012). The growing production of biodiesel has made the excess of glycerol in the market, so the price of glycerol decreases. In fact, glycerol can be used in many applications for example pharmaceutical and oral care, textile industry, plastic industry, polymer industry and livestock feed. One of the value added way of glycerol is to be used as the carbon and energy sources for microorganisms (Alvarez et al., 1996; da Silva et al., 2009). *R. opacus* PD630 is an oleaginous bacterium that grown on gluconate medium is capable of accumulating TAGs accounting for up to 76% of the cell dry weight (CDW) (Wältermann et al., 2000). Nevertheless, *R. opacus* PD630 can consume glycerol and convert to triacylglycerol (TAGs) and other products. This study investigated the growth characteristic of *R. opacus* PD630 cultivated on glycerol based MSM medium. The success of this cultivation will be the alternative glycerol utilization in the future.

Methodology

Bacterial strain and media

R. opacus PD630 (DSM 44193) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany). Nutrient broth (NB) and purified glycerol (10%w/v) with mineral salt medium (MSM) (Schlegel, 1961) were used as culture media, but changed 1 g ammonium chloride to 7.74 g ammonium acetate in MSM.

The medium per 985 mL contains 2.9 g $Na_2HPO_2{\cdot}2H_2O$, 2.3 g KH_2PO_4, 7.74 g CH_3COOCH_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 g $NaHCO_3$, 0.01 g $CaCl_2{\cdot}2H_2O$, ferric ammonium citrate solution 20 mL and 5 mL trace elements solution SL-6 (Pfennig, 1974). Trace elements contain 0.5 g/L $MnCl_2{\cdot}7H_2O$, 0.3 g/L H_3BO_3, 0.2 g/L $CoCl_2{\cdot}6H_2O$, 0.1 g/L $ZnSO_4{\cdot}7H_2O$, 0.03 g/L $Na_2MoO_4{\cdot}2H_2O$, 0.02 g/L $NiCl_2{\cdot}6H_2O$, 0.01 g/L $CuCl_2{\cdot}2H_2O$. Media were sterilized by autoclaving at 121 °C for 15 minutes. The strain was routinely maintained on NB agar medium (8%w/v) and stored at 4°C.

Preparation of seed culture and culture conditions

A loopful of cells from a single colony grown on NA plate at 30°C for 3 days was used to inoculate in 5 mL of NB in the test tube. The cultured tube was incubated in shaking incubator (200 rpm) at 30°C for 24 hours until it reached an optical density (OD) of approximately 0.65 at 660 nm, as determined by a spectrophotometer (Anthekie Advanced, Secomam, France). Then transferred 25 µL of cultured broth from the test tube to 50 mL of NB in 250 mL Erlenmeyer flask for scaling up. It was incubated for 27 hours in shaking incubator (200 rpm) at 30°C and used as the inoculum. Twenty five percentage of inoculum was added to 50 mL of purified glycerol (10%w/v) with MSM in 250 mL Erlenmeyer flask.

Data analysis

Cell growth was estimated by measuring the optical density at 660 nm or the dry cell weight (DCW). The DCW was determined by harvesting approximately 10 mL of the cell suspension and centrifuge three times at 6000×g for 15 minutes and washed the bacterium cell once in distilled water. The supernatants of the culture broth were also used to analyze the glycerol and acetic acid concentration after filtration through 0.2 µm-pore-size filter. Glycerol and acetic acid concentrations were measured by high performance liquid chromatography (HPLC, Spectra-Physics) fitted with an Aminex HPX-87H column (300mm×7.8mm, BIO-RAD) coupled to a refractive index (RI) detector. The column was eluted with 5 mM H_2SO_4 as mobile phase at 50 °C and a flow rate of 0.6 mL/min.

Results and discussion

Growth of *R. opacus* PD630 on nutrient broth.

Growth of *R. opacus* PD630 on NB was measured every 3 hours until 69 hours of the cultivation time and determinated dry cell weight and optical density at 660 nm. There was a relationship between dry cell weight and OD_{660} as linear function showed in Equation 1.

$$Y = 1.3048X - 0.0355 \quad (1)$$

where Y is OD_{660} and X is DCW (g/L). Data analysis of 0.9857 regression coefficient (R^2) 0.9857 suggested that the quadratic equation was the appropriate model (Figure 1).

Growth pattern of *R. opacus* PD630 on NB showed the short lag phase after 15 hours of the cultivation. The biomass increased rapidly from 0.076 g/L to 1.314 g/L after 36 hours of the cultivation and the maximum biomass was obtained. In general, the biomass reached the maximum values and showed no difference after 36 hours of cultivation. The exponential phase of growth started from 15 hours to 36 hours of the cultivation time. While, at 27 hours of the cultivation time was chosen as a middle log phase and used as the inoculum time (Figure 2).

Growth of *R. opacus* PD630 on MSM with 10%w/v of purified glycerol.

Growth of *R. opacus* PD630 on MSM with 10%w/v of purified glycerol was measured every 24 hours of the cultivation time and analyzed dry cell weight and optical density at 660 nm. There was a relationship between dry cell weight and OD_{660} as linear function as showed in Equation 2.

$$Y = 0.6461X + 0.0687 \quad (2)$$

where Y is OD_{660} and X is DCW (g/L). Data analysis of 0.9825 regression coefficient (R^2) suggested that the equation was the appropriate model in quadratic form (Figure 1).

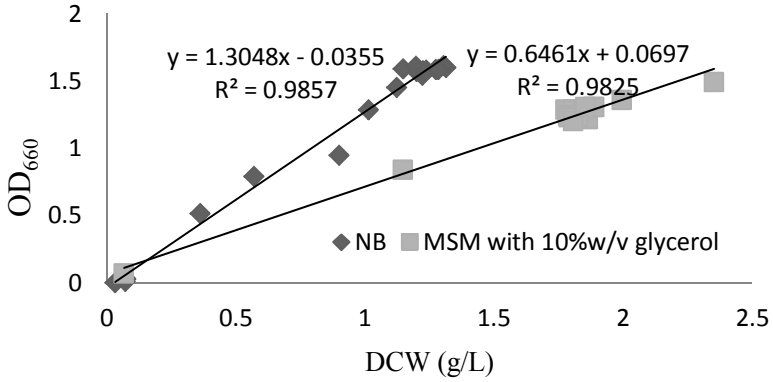

Figure 1: Relationship between growth (OD_{660}) of *R. opacus* PD630 and cell dry weight (CDW) on NB and MSM with 10%w/v of purified glycerol.

As showed in Figure 2 and Figure 3, the profiles showed two carbon sources; acetic acid from ammonium acetate and glycerol; in this cultivation. Glycerol was chosen in this work because the previous work reported that it produced both of TAG and β-carotene when cultivated on MSM and glycerol (Sinprasertchok, 2013). The biomass increased rapidly from 0.068 g/L to 1.883 g/L after 2 days of the cultivation with short lag phase. After ammonium acetate was consumed, the cells looked like it was going to the stationary phase. However, the biomass increased again after 12 days of the cultivation because it consumed the second carbon source after 14 days of the cultivation and increased from 1.856 g/L to 2.353 g/L.

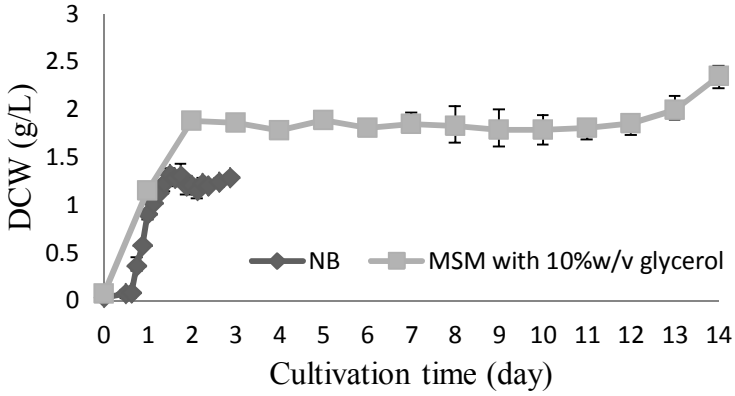

Figure 2: Growth characteristic of *R. opacus* PD630 on MSM and 10%w/v of purified glycerol.

In this cultivation, ammonium acetate was recognized as carbon source (acetate or acetic acid) and nitrogen source (ammonium). Acetate concentration can be analyzed in acetic acid form according to Venkataramanan et al. (2012). As be seen in Figure 3, Acetic acid concentration has decreased dramatically in the first two days which responded well with the increasing of biomass. Ammonium acetate 7.74 g/L in MSM was used in this study, which was high enough for the bacterial growth for 2 days.

As shown in Figure 3, glycerol concentration is constant at 10%w/v for 11 days of the cultivation time and reduced rapidly from 10%w/v to 5.54%w/v during 11th to 14th day. When the biomass started to increase, glycerol concentration started to decrease. This result is the same as Sinprasertchok (2013) who reported that acetic acid from ammonium acetate supported growth. Alvarez et al. (1996) also reported the utilization of glycerol by *R. opacus* PD630.

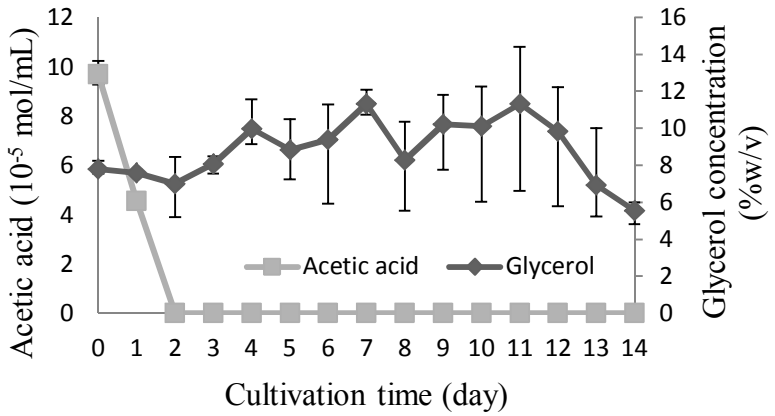

Figure 3: The remaining acetic acid and glycerol concentration in the cultivation of *R. opacus* PD630 on MSM and 10%w/v of purified glycerol.

From this experiment, it is obvious that MSM with high concentration of nitrogen source and glycerol was not a proper carbon source to cultivate *R. opacus* PD630 because it took long time before consuming glycerol. If glycerol will be used as carbon source, the researcher should activate the cells or modify the medium to allow better glycerol utilization.

Conclusion

In summary, *R. opacus* PD630 could utilize glycerol as a carbon source, but it took long time. Growth characteristic of the cells in this work was caused by two carbon sources; acetic acid from ammonium acetate and glycerol. This medium at higher nitrogen source concentration in this study could not support *R. opacus* PD630 to produce β-carotene as in Sinprasertchok's work (2013). Future study has to be done if glycerol is still used as carbon source for β-carotene cultivation.

Acknowledgements

This project is supported by the faculty of engineering, Kasetsart University.

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