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THE GROWTH RATE OF HAEMOPHILUS PARAGALLINARUM PROFILE IN SHAKE FLASK FERMENTATION; DETERMINATION [SUBSTRATE]$_{CRITICAL}$

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Abstract. Determination [substrate] $_{critical}$ is important in a fermentation process especially for optimizing coryza vaccine production. The objectives of the study were to establish the effect of different substrates on the production of *Hpg* cell and to study the effect of different concentration of a selected carbon sources on the specific growth rate of *Hpg*. The data obtained indicate that fructose produced the highest concentration of cell biomass of *Hpg*, closely followed by maltose, lactose, glucose, and sucrose. However, from operating cost point of view, glucose was selected as the most suitable carbon source due to its low cost and acceptable *Hpg* cell yield. The critical glucose concentration was found to be at 0.5 %, which corresponds to specific growth rate *Hpg* of 0.4 h^{-1}.

Keywords: *Haemophilus paragallinarum (Hpg)*, critical substrate, specific growth rate

Abstrak. Penentuan kadar [substrat] $_{kritikal}$ adalah sangat penting dalam proses fermentasi terutama di dalam pengoptimuman pengeluaran vaksin coryza. Objektif kajian ini adalah untuk mengkaji kesan substrat yang berbeza ke atas penghasilan sel *Hpg* dan kesan perbezaan kepekatan sumber karbon yang dipilih bagi kadar pertumbuhan spesifik *Hpg*. Berdasarkan keputusan yang diperolehi, didapati fruktosa telah menghasilkan kepekatan biojisim *Hpg* yang tertinggi diikuti oleh maltosa, laktosa, glukosa, dan sukrosa. Walau bagaimanapun untuk mendapatkan kos operasi yang rendah, glukosa telah dipilih sebagai sumber karbon yang sesuai, serta ia mampu memberikan hasil yang memuaskan dalam penghasilan sel *Hpg*. Kepekatan glukosa yang kritikal didapati pada 0.5%, sejajar dengan kadar pertumbuhan *Hpg* pada 0.4 per jam.

Kata Kunci: *Haemophilus paragallinarum (Hpg)*, substrat kritikal, kadar pertumbuhan spesifik

1.0 INTRODUCTION

Medium formulation is an essential stage in the design of successful laboratory experiments, pilot scale development and manufacturing processes. The constituents of a medium should satisfy the elemental requirements for cell biomass and metabolite production and adequate supply of energy for biosynthesis and cell maintenance.

It is a common practice to use carbohydrates as the carbon source in microbial fermentation processes. The type of carbon source often influences the formation of biomass, production of primary and secondary metabolites [1,2]. Fast growth due to high concentrations of rapidly metabolized sugars is often associated with low productivity of secondary metabolites.

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The objectives of the study were to establish the effect of different substrates on the production of *Hpg* cell for coryza vaccine production and to study the effect of different concentration of a selected carbon sources on the specific growth rate of *Hpg*.

2.0 MATERIALS AND METHODS

2.1 Medium Preparation and Microorganism

The medium and agar plate consisted of Peptone, NaCl, glucose, $Na_2HPO_412H_2O$, NADH, chicken serum as described in Mel, et al. [3]. The chicken serum and NADH were sterilized separately using 0.45 mm membrane filter. The final pH of the medium was adjusted at 7.3 with NaOH (1N) and HCl (1N).

The strain used was *Haemophilus paragallinarum* (*Hpg*) 221 obtained from Veterinary Research Institute (VRI), Ipoh, Perak. The agar plate was incubated at 37°C for 24 hours. A single colony from agar plate was transferred into universal bottle containing 10 ml standard medium. This bottle was then incubated at 37°C for 18 hours. One ml culture cell from the bottle was added into a universal bottle containing 9 ml medium as the inoculum for the shake flask fermentation. All the steps were carried out under aseptic condition. The inoculum culture was incubated at 37°C for 10 hours. A similar procedure was followed for the preparation of 100 ml medium for shake flask fermentation.

The five shake flasks containing different carbon sources (glucose, sucrose, fructose, maltose and lactose) were prepared. 10ml culture was then transferred into each of the shake flasks. The inoculated flasks were then incubated in an incubator shaker at 37°C, 150 rpm and for 16 hours. 10ml sample was taken out from each shake flasks and transferred into separate bottle. These samples were then analyzed for cell dry weight.

The medium with chosen substrate of different concentrations (0%, 0.5%, 1.0%, 1.5% and 2%) were prepared in shake flask. 10 ml inoculum from each of the universal bottles were transferred into shake flasks. These shake flasks were incubated in an incubator shaker at 37°C and 150 rpm. Samples were withdrawn at various time interval for analysis.

2.2 Analytical Methods

Cell growth was determined by monitoring the optical density (OD) of the fermentation broth using a spectrophotometer. The OD was measured at 660nm using 3 ml of broth sample in a 4.0 ml glass cuvette. In parallel, the cell dry weight was determined by centrifuging the samples at 6000 rpm for 30 minutes. The supernatant was discarded and pellet was resuspended in distilled water and placed into weight cap. The sample was dried in an oven at 80°C overnight and cooled in a dessicator before weighing.

2.3 Determining the Specific Growth Rate (μ)

Cell division occurs in the exponential phase. The rate of cell number (N) increase is proportional to the number of cells. Cell increase in a geometric progression 2^0, 2^1, 2^2,.2^m after m divisions. For example, if the initial cell number was N_o, the number after m generations is $2^m N_o$.

Instead of cell number, it is more convenient to use cell dry weight per volume X as a measure of cell concentration. During the exponential phase in a batch reactor, it could be written as $\mu X = dX/dt$ where, m is the *specific growth rate* of the cell. The above equation can be integrated from the end of the lag phase ($X = X_o$, $t = t_{lag}$) to any point in the exponential phase (X,t) where, $X = X_o e^{\mu(t-tlag)}$ or $\ln(X/X_o) = \mu\,(t - t_{lag})$.

3.0 RESULTS AND DISCUSSION

3.1 Selection of the Suitable Sugar for *Hpg* Growth in Shake Flask

The most suitable sugar as carbon source for *Haemophilus paragallinarium* growth was investigated. The study was carried out using five types of sugar such as sucrose, maltose, fructose, glucose and lactose. As observed, each of the substrate generated a different cell concentration after 16 hours incubation. The experiment was carried out in duplicate. Figure 1 shows that fructose was the best substrate producing highest cell concentration (2.52 g/l) whereas maltose yielded a slightly lower growth of *Haemophilus paragallinarium* (2.38 g/l). Sucrose as carbon source for *Haemophilus paragallinarium* produced the lowest amount cell (1.82 g/l). The lowest cell produced may be due to component of sucrose used in fermentation, the sucrose must be firstly hydrolized

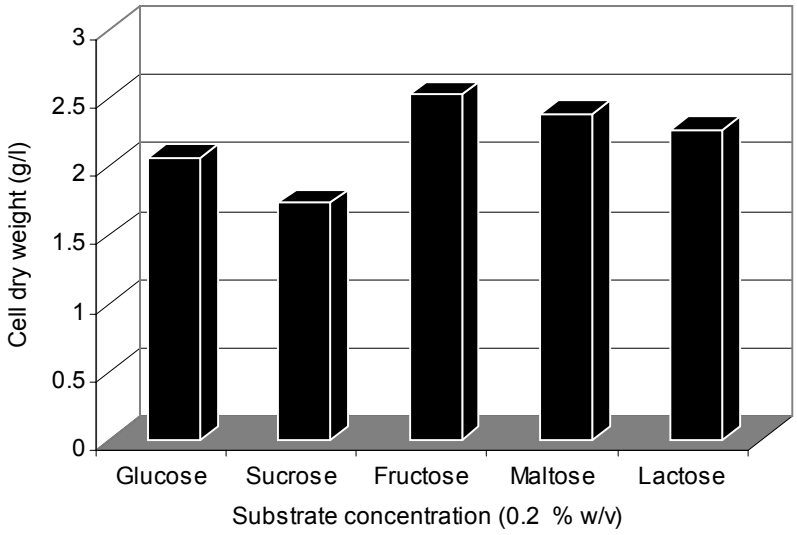

Figure 1 Effect of different substrate on the *Hpg* growth

into simple sacharide (glucose and fructose). Due to cost factor, fructose was not selected. Hence, glucose was selected as a main carbon source. Glucose is widely available with lower cost compared to fructose.

3.2 Effect of Different Glucose Concentrations on Growth Rate of *Hpg*

The growth profile of *Haemophilus paragallinarium* in different glucose concentrations (0%, 0.5%, 1.0%, 1.5% and 2%) was investigated (Figure 2). The graph shows that at different glucose concentrations, different specific growth rates were observed. The calculated μ, each of the different glucose concentration has a significant influence on cell specific growth rate. The graph μ versus percentage of glucose concentration is shown in Figure 3. This graph is similar to the profile from relation of μ and concentration of substrate. The curve is a positive slope (at the beginning to a maximum point), and gradually changes to negative. This maximum point was called $[S]_{critical}$. From the result, it was noted that the consumption of glucose would produce specific growth rate less than that of the optimum rate. This effect is called a glucose effect or a Crabtree effect [4,5]. Glucose is the substrate that gives the complete effect to *Haemophilus paragallinarum* growth. Glucose effect on this facultative anaerobic bacteria is confirmed [3]. So, glucose effect could be the reason for the reduction of specific growth rate when glucose concentration increased to 2.0%. The growth was inhibited at this concentration.

The existence of excess toxic would inhibit the essential internal and external process, which could kill the cell. It was concluded that glucose effect should be avoided

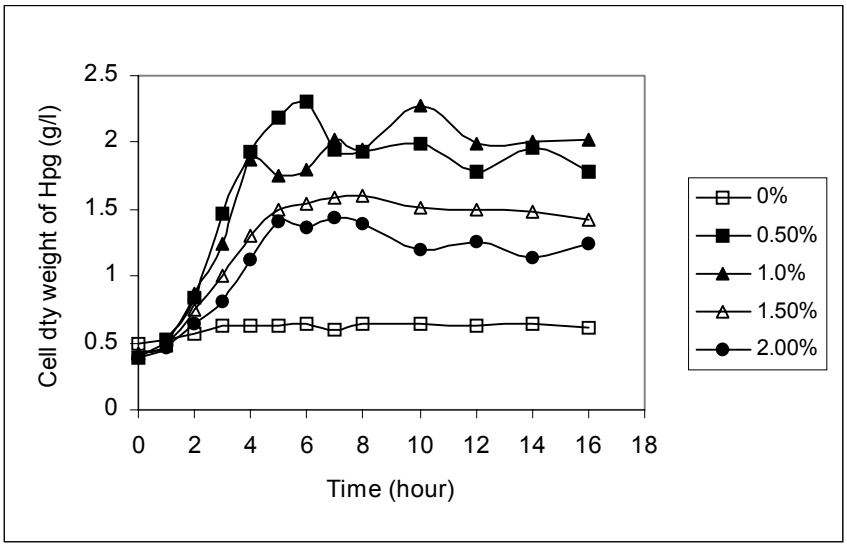

Figure 2 Growth profile of *Hpg* in different glucose concentration

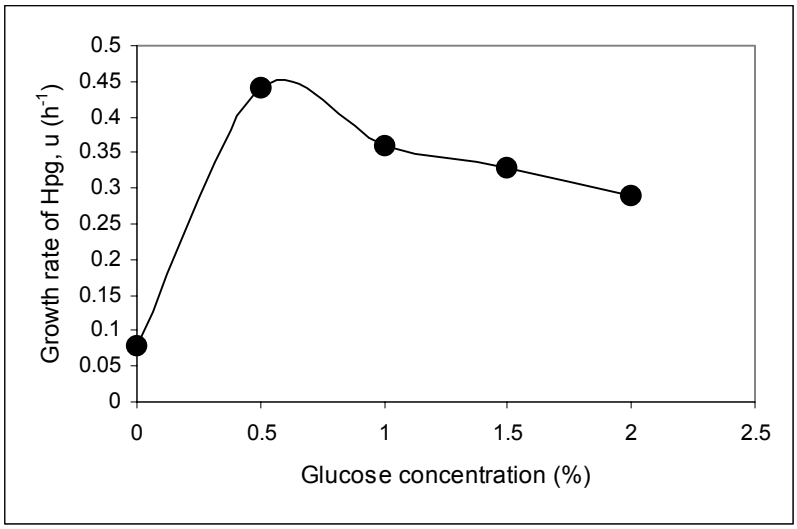

Figure 3 $[\text{Glucose}]_{\text{critical}}$ on *Hpg* growth rate

in order to produce high cell density. Thus, from the result obtained, it is suggested that a medium with a lower concentration of glucose than $[S]_{\text{critical}}$ to be used in the next experiment. The $[S]_{\text{critical}}$ obtained from study occurred at glucose concentration of 0.5%, correspond to the specific growth rate, μ at 0.44 h^{-1}. This value correlate to the result in the previous work [3] that the glucose concentration was selected in the range of 0.1% to 0.5%.

4.0 CONCLUSION

Glucose, fructose, maltose and lactose were shown to be potential carbon source in *Haemophilus paragallinarum* fermentation carried out in shake flasks. However, glucose was chosen to be the most suitable substrate for *Hpg* fermentation since it was metabolized at acceptable rate and cheap. The excess of glucose concentration has a negative influence on *Hpg* growth. The value of glucose concentration should not exceed $[S]_{\text{critical}}$ of 0.5%. The growth rate reached an optimum 0.44 h^{-1} at critical glucose concentration.

SYMBOLS

m	specific growth rate
N	cell number
N_o	initial cell number
X	cell concentration in dry cell weight
X_o	initial cell concentration

t time course of fermentation

t_{lag} fermentation time at lag phase

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