Intrinsic growth and substrate utilization kinetics parameters for *Oscillibacter* sp., a CO-utilizing acetogenic bacterium

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Abstract

Despite the fact that Carbon monoxide (CO) is toxic to humans and other species, it is used as a sole carbon and energy source by anaerobic microorganisms called as "acetogens", and produce energy dense fuels and useful chemicals. Thus the biological conversion of CO and H_2 rich synthesis gas (syngas) has been an area of interest for the researchers in the recent decades. However, the low solubility of CO and its high affinity to metal ions in the CO oxidizing enzymes are the big hurdles to achieve a successful operation of commercial sized bioreactor. Hence it is desired to find the optimum concentration of the CO that do not inhibit the microbial growth and efficiency of CO oxidizing enzymes in the fermentation media.

The aim of this study was to evaluate the CO utilizing capacity for a newly isolated strain which is identified as *Oscillibacter sp.* Cow-5. This strain produces various chemicals: acetate, butyrate and small amount of ethanol and iso-valerate from CO under strict anaerobic conditions. The strain was grown in batch reactor under optimum growth conditions and a modified Monod equation was used to determine the maximum growth rate (μ_{max}) and Monod constant (K_s). Simulated data were well fitted with experimental one, and two parameters, μ_{max} and K_s, were estimated as 0.13h^{-1} and 0.08mM CO, respectively using a nonlinear second order regression. Meantime, the apparent dissolved CO inhibition has been found at 0.94mM CO (≈1.16atm of PCO) from the plot of C* vs. C*/μ.

Introduction

Syngas fermentation has gained a widespread interest as an alternative to produce energy dense fuels and valuable chemicals . It can help in the reduction of environmental contaminants (CO and CO_2) with the added benefit of the generation of biofuels and valuable chemicals. Most of the research that has been conducted in this field have revealed that the inhibition in the fermentation media is one of the main hurdles in the in the commercialization of the microbial conversion of gaseous substrates. This inhibition could be caused by the toxic nature of the substrates and/or by the products and other contaminants. As a result microbial growth, substrate utilization and product formation rates are slowed down or even inhibited. Substrate inhibition occurs during the initial oxidation of the electron donor substrates. It reduces or stops the oxidation of electron donor either by competitive or non-competitive inhibition resulting in the slow uptake rates. (Shuller and Kargi, 2010) Furthermore the intracellular reactions that require electrons and ATP will be slowed due to reduction in the electron flow.

Carbon monoxide (CO) which is the main component of syngas is toxic to the most of the living organisms. However, several microorganisms utilize CO as a sole carbon and energy source. For instance "acetogens" grow chemoautrophically on CO and produce useful fuels and chemicals. However, due to high affinity of CO to metal ions, it binds with the cofactors of the CO oxidizing enzymes and reduces their activity by non-competitive inhibition. (Vega, Clausen et al, 1989) Thus the higher concentrations of CO in the fermenters inhibit the microbial growth and substrate utilization rate. Same was presented by (Chang, Kim et al, 2001). Hence it is strongly desired to find the optimum concentrations of dissolved CO to design and control large scale fermenters. The focus of this study was to find the optimum range of dissolved CO that do not inhibit the growth and CO consumption rate of newly isolated strain *Oscillibacter sp.* Cow-5.

Experimental Section

Microbial Strain: **This study used a pure in-house bacterial strain identified as *Oscillibacter sp.* Cow-5.**

Growth Media Composition: ***Oscillibacter sp.* Cow-5 is cultivated in Phosphate buffered basal medium (PBBM). Media composition was adopted from (Chang, Kim et al, 1998).**

Measurement of Specific Growth Rate: **Specific growth rate (μ) by using the following formula:** $\mu(h^{-1}) = \frac{1}{x}\frac{dx}{dt}$

Dissolved CO: **Dissolved CO concentrations (mM) at various partial pressures were measured by using Henry law ($C^*=P_{CO}/H$).**

Estimation of Growth and Intrinsic Kinetic Parameters

Triplicate values of dissolved CO concentrations (C^*) were plotted against (C^*/μ). Growth and intrinsic kinetics parameters were then determined by using second order nonlinear regression of Andrews's equation.

Results

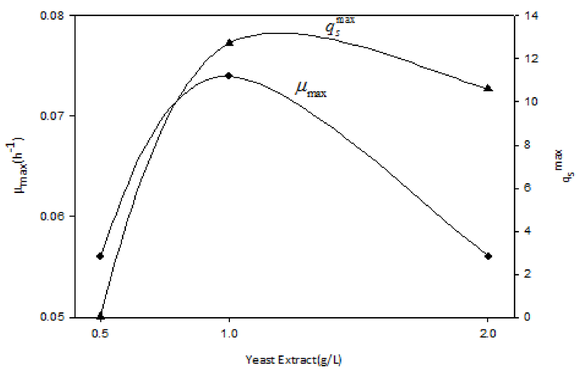
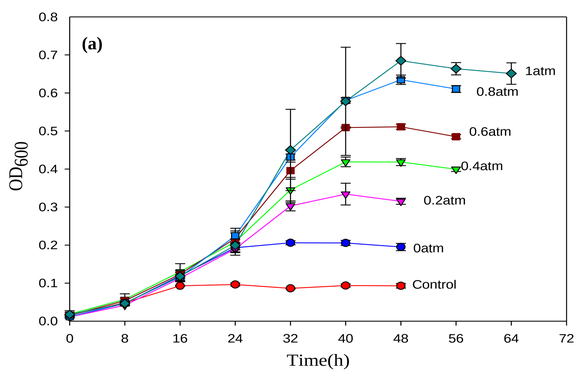


Fig.1 Maximum specific growth rate and specific CO utilization rate at different concentrations of yeast. Each data point is the mean of triplicate values.



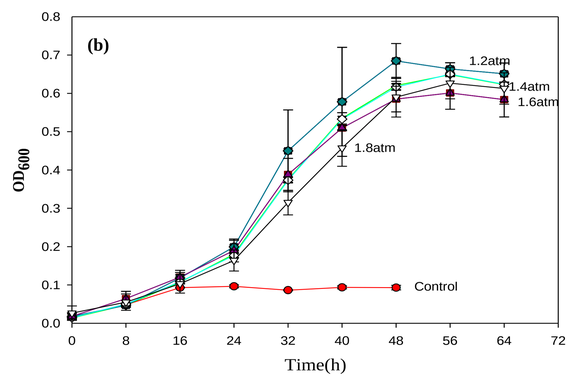


Fig.2(a) Growth curve at 0 to 1atm. **Fig.2**(b) Growth curve at 1.2 to 1.8atm. Each data point is the mean of triplicate values.

Conclusions

1. **The optimum requirement of yeast extract for *Oscillibacter* sp. Cow-5 is 1g/L**
2. **The estimated growth and kinetics parameters are presented in table 1. The best dissolved CO operating condition is 0.55mM (equivalent to 0.7 atm)**
3. **Apparent dissolved CO inhibition is found at around 0.94mM(equivalent to 1.16atm)**
4. **Optimum pressure range for CO fermentation using *Oscillibacter* sp. Cow-5 is 0.7 to 1.1atm.**

References

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Parameters	Value
μ_{max}	0.13h^{-1}
K_s	0.086mM
K_i	3.53mM
$(K_sK_i)^{1/2}$	0.55mM

Tab. 1 Growth and intrinsic kinetics parameters

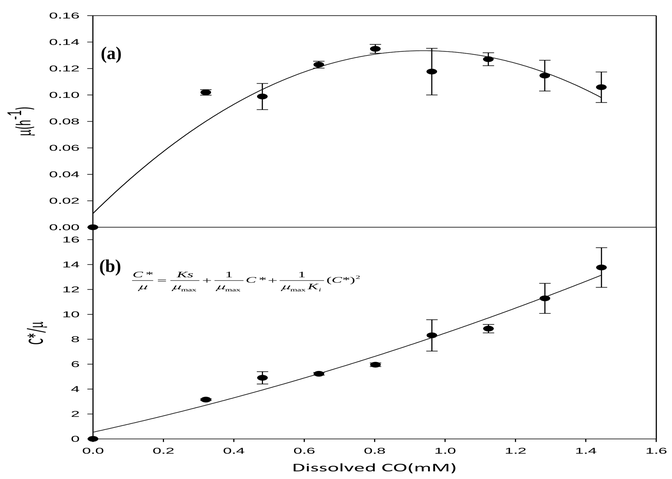

Fig.3(a) Specific growth rate(μ) against dissolved CO.

Fig.3(b) Dependence of growth on dissolved CO using Andrews model equation.

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