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Gas mass transfer with microbial CO_2 fixation and poly(3-hydroxybutyrate) synthesis in a packed bed bioreactor

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ABSTRACT

Gas mass transfer is the rate-limiting step in CO_2 fixation by a hydrogen-oxidizing bacterium *Cupriavidus necator* under chemolithoautotrophic conditions. The mass transfer rates (i.e. the gas uptake rates) were measured in a packed bed bioreactor under the conditions of microbial growth on CO_2. The k_La values of O_2 and H_2 were determined under either O_2 or H_2 limitation, respectively, and the ratio was proportional to a square root ratio of the molecular diffusivities in water, indicating that a surface renew model is suitable for the gas mass transfer in the packed bed. At a low gassing rate, the O_2 mass transfer coefficient was to different extent affected by packing materials, bed geometry, liquid velocity, and liquid distribution. The liquid distributor and/or bed void space exhibited a high effect on the mass transfer coefficient. With microbial growth, the gas fermentation was becoming limited either by dissolved O_2 or H_2, depending on gas composition, but neither O_2 nor H_2 limitation could trigger the formation of a substantial amount of poly(3-hydroxybutyrate) (PHB) under nutrient-rich conditions. Instead, when a nitrogen nutrient control strategy was applied, PHB synthesis was significantly improved and the PHB content reached up to 67 wt% of cell mass.

1. Introduction

Poly(3-hydroxybutyrate) (PHB) is a biodegradable and biocompatible polyester for a variety of applications such as eco-friendly packaging [1], tissue engineering [2], and catalytic refining for bio-based chemicals and fuels [3]. PHB is currently produced through microbial fermentation on agricultural products including sugar, starch and vegetable oil [4–6], which has raised concerns on its sustainability [7]. New technologies have been researched to produce PHB and other polyhydroxyalkanoates (PHA) from inexpensive feedstocks including various wastes [8–10]. CO_2 from fossil fuel combustion is a prime greenhouse gas and has attracted renewed interest as a carbon source in production of PHB and other bio-products [11,12]. Some hydrogen-oxidizing bacteria can fix CO_2 by using H_2 and O_2 in autotrophic dark conditions [13–15] and accumulate a large amount of PHB as a carbon and energy reserve material [11,16]. Because of the low solubility of the gas substrates in aqueous solution [17], however, mass transfer of gas molecules to microbial cells is a rate-limiting bottleneck and a technical challenge to PHB production from CO_2. A bioreactor with high mass transfer coefficient (k_La) is a prerequisite for the gas fermentation with a high cell density and high PHB productivity [16].

Packed beds have been widely used in industries for gas absorption because of low energy consumption, high gas/liquid contact area and mass transfer rate [18]. A typical packed bed is a column filled with solid packing materials, and a liquid stream is spread on the bed, moving downward with a co- or counter-current gas flow through the bed [19]. Liquid films on the packing material's surface are continuously renewed, facilitating gas-liquid contact and mass transfer [20]. Various packing materials with different material properties and geometric structures are available to form beds of unique properties, including surface hydrophobicity, liquid holdup, local hydrodynamics, void space, pressure drop, effective surface area per bed volume, and so on [21]. The bed performance and mass transfer rate are also affected by the liquid distributer as well as the gas and/or liquid velocities [22]. Packed bed bioreactors have been used in treatment of wastewater or waste gas as biofilters or bioscrubbers [23–25]. Little work, however, has been done in microbial cultivation on gas substrates. In contrast to a conventional aerated bioreactor, a packed bed bioreactor for gas fermentation can be operated at very low gassing rates to avoid wastage of un-used gas. The medium solution is recirculated via a pump to provide continuous renewal of liquid films on the packing materials as well as appropriate liquid mixing for pH and temperature control. According to the two-film theory, the overall mass

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Nomenclature

k_L	Gas mass transfer coefficient in liquid phase [L m^{-2} h^{-1}]
k_La	Volumetric mass transfer coefficient [h^{-1}]
$(k_La)_{O2}$	Overall volumetric mass transfer coefficient of oxygen [h^{-1}]
$(k_La)_{H2}$	Overall volumetric mass transfer coefficient of hydrogen [h^{-1}]
OUR	Oxygen uptake rate [mole L^{-1} h^{-1}]
HUR	Hydrogen uptake rate [mole L^{-1} h^{-1}]
N	Oxygen flux [mole m^{-2} h^{-1}]
A_e	Gas-liquid interfacial area [m^2]
V_L	Liquid volume [L]
a	Effective gas/liquid interfacial area per liquid volume [m^2 L^{-1}]
P_{O2}	Oxygen Partial pressure [atm]
$C^*_{O2,L}$	Equilibrium concentration of oxygen under corresponding partial pressure [mole L^{-1}]
$C_{O2,L}$	Dissolved oxygen concentration in liquid solution [mole L^{-1}]
$F_{in,O2}$	Oxygen molar flow rate at the inlet of bioreactor [mole h^{-1}]
$F_{out,H2}$	Oxygen molar flow rate at the outlet of bioreactor [mole h^{-1}]
$P_{in,O2}$	Oxygen partial pressure at the inlet of bioreactor [atm]
$P_{out,O2}$	Oxygen partial pressure at the outlet of bioreactor [atm]
D_L	Molecular diffusion coefficient of a gas in liquid [cm^2 s^{-1}]
V	Turbulence velocity [m s^{-1}]
L	Length [m]
D_{LH2}	Diffusion coefficient hydrogen [cm^2 s^{-1}]
D_{LO2}	Diffusion coefficient oxygen [cm^2 s^{-1}]
μ_{max}	Maximum specific growth rates [h^{-1}]

transfer resistance of gas absorption in water consists of the mass transfer resistances in the gas phase and the liquid phase. For the gases of very low solubility in water such as H_2 (0.78 mmole L^{-1} under 1 atm, 25 °C) and O_2 (1.26 mmole L^{-1} under 1 atm, 25 °C), the mass transfer resistance in liquid phase is predominant and hence the overall mass transfer rate is close to the mass transfer rate in the liquid phase [26]. The utilization of dissolved gas by microbial cells in the medium solution could enhance the gas mass transfer rate. It is therefore useful to measure the gas mass transfer rates in the medium solution of microbial CO_2 fixation. This "on-site" measurement of mass transfer coefficients (k_La) generates new knowledge on the mass transfer of sparingly soluble gases in packed bed bioreactors.

In this work, the microbial gas consumption under a quiescent gas phase was measured when the microbes were directly exposed to the gas substrates or immersed in a medium solution. A bench-top packed bed bioreactor was operated at a low gassing rate to measure the steady state uptake rates of H_2 and O_2, respectively, from which the mass transfer coefficients were determined. Random and structured beds of different materials, size and geometry were evaluated at the low gassing rate in a broad range of liquid velocity. Two types of liquid distributors (shower versus nozzle sprayer) were tested for their effects on O_2 mass transfer coefficient and the overall performance of gas fermentation. Finally, the packed bed bioreactor was operated under nutrient control to demonstrate PHB production from CO_2.

2. Materials and methods

2.1. Media and cultures

A strain of hydrogen-oxidizing bacterium *Cupriavidus necator* (*C. necator*) isolated from local environment was maintained in the laboratory and used in this work [27]. The strain was activated at 30 °C in a nutrient medium solution (5 mL) that contained (1L): 10 g yeast extract, 10 g peptone, 5 g meat extract, and 2 g $(NH_4)_2SO_4$. The nutrient culture was used as the inoculum for cultures in a mineral solution that contained (1L): 2.4 g KH_2PO_4, 2.5 g $Na_2HPO_4{\cdot}2H_2O$, 2 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4{\cdot}7H2O$, 0.5 g $NaHCO_3$, 0.1 g ferric ammonium citrate, and 1 mL trace element solution. The trace element solution contained (1 L): 0.6 g H_3BO_3, 0.4 g $CoCl_2{\cdot}6H_2O$, 0.2 g $ZnSO_4{\cdot}7H_2O$, 0.06 g $MnCl_2{\cdot}4H_2O$, 0.06 g $NaMoO_4{\cdot}2H_2O$, 0.04 g $NiCl_2{\cdot}6H_2O$, and 0.02 g $CuSO_4{\cdot}5H_2O$. The mineral solution was sterilized with a bottle-top filter (0.2 μm). The mineral agar was prepared by adding 15 g agar in 1 L mineral solution above and autoclaved at 121 °C for 10 mins.

2.1.1. Jar culture

Mineral agar or solution (25 mL) in Petri dishes (8.5 cm diameter (Φ)) was inoculated and left in an anaerobic jar (2.5L). The jar was flushed with a gas stream of 70% H_2, 20% O_2, and 10% CO_2 till constant gas composition that was monitored with an O_2/CO_2 gas analyzer (Tandem PRO, Magellan, UK). The jar was sealed and incubated at 30 °C. The gas pressure was monitored and recorded with time.

2.1.2. Bottle culture

100 mL mineral solution in a heavy duty plastic bottle was sterilized at 121 °C for 10 mins and inoculated with 5 mL inoculum above. The bottle was flushed with a gas stream of 70% H_2, 20% O_2, and 10% CO_2 described above and sealed. The bottle was shaken in a rotary incubator of 30 °C at 200 rpm and reflushed with the gas mixture every 48 h till the optical density (OD) reached 4. Harvested by centrifugation at 5000g for 5 mins, the cells were washed with distilled water twice and re-dispersed in 100 mL mineral solution to start gas fermentation in a packed bed bioreactor.

2.2. Packed bed bioreactor

Fig. 1 is the schematic structure of the bench-top bioreactor used in this study. The top part of the reactor was a glass column (6 cm inner Φ × 23 cm height). Different packing materials (see Table 1) were poured into the column to form randomly packed beds supported by a ceramic mesh at the bottom. Structured beds were directly put in the column (Table 1). A perforated liquid distributor (25 × 1.0–1.5 mm holes) was installed 50 mm above the beds. The bottom part of the reactor was a 3 L spherical glass vessel filled with 500 mL medium solution at the beginning of gas fermentation. The solution was agitated at 150 rpm on a magnetic agitation hot plate (Super-Nuova™ Single-Position Digital Stirring Hotplates, Thermo, USA) with temperature control in precision of 0.1 °C. The temperature in the bottom vessel and the top column was maintained at 35 and 32 °C, respectively. The dissolved O_2 (DO_2) and H_2 (DH_2) concentrations were monitored with an optical DO_2 meter (ProODO, YSI, USA) and Clark-type DH_2 microsensor (H_2-NPLR and amplifier, Unisense A/S, Denmark). The solution pH was controlled at 6.8 ± 0.1 by adding a base solution (5% NH_4OH for cell growth or 2 M NaOH for PHB accumulation) through a pH controller (DLX Solenoid pump, North West Fluids Control Inc, Canada). The medium solution was circulated with a peristaltic pump (7523-20, Cole–Parmer, Vernon, IL, USA) at flow rates from 0.1 to 1.2 L min^{-1}, or liquid velocities from 2 to 26 m h^{-1}. The liquid velocity was calculated from the flow rate divided by the cross section area of the packed bed.