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Dissolved carbon dioxide concentration profiles during very-high-gravity ethanol fermentation

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ABSTRACT

The dissolved carbon dioxide (DCO_2) evolved from *Saccharomyces cerevisiae* was measured for the duration of very-high-gravity (VHG) ethanol fermentation conducted at four glucose feeds (150, 200 ± 0.21, 250 ± 0.12, and 300 ± 0.28 g/L). The DCO_2 concentration profiles depict evolution patterns that depend on the extent of glucose utilized. A mass balance for DCO_2 was proposed by taking into account the physiochemical behavior of CO_2 and biological processes occurring under VHG ethanol fermentation conditions. The proposed equations could interpret the underlying CO_2 desorption, CO_2 conversion, and CO_2 evolution on the basis of yeast activity during VHG ethanol fermentation. Consequently for 150 and 200 ± 0.21 g glucose/L absence of yeast activity was interpreted in terms of zero CO_2 evolution rate ($CER(t)$). In contrast, under 250 ± 0.12 and 300 ± 0.28 g glucose/L conditions the presence of non-zero residual glucose resulted in non-zero $CER(t)$. The mass balance equation could also interpret that the physical desorption of CO_2 from the fermentation broth did not follow the equilibrium relation between dissolved and off-gas CO_2 concentrations as defined by Henry's law under current experimental conditions.

1. Introduction

During ethanol fermentation, yeast utilizes glucose as both a carbon and an energy source, producing ethanol and carbon dioxide (CO_2) as key metabolites. The role of CO_2 in microorganism metabolism has been discussed by several authors [1–6]. Due to the stoichiometric relationship between substrate utilization, ethanol production, and yeast growth, the CO_2 evolved is a direct measure of yeast activity in the fermentation broth. Hence, monitoring CO_2 concentration should enable monitoring of the fermentation process in an inexpensive manner [5,8–11]. However, the CO_2 evolved during fermentation remains in dissolved form in the fermentation broth and is transferred to the gas phase through various mass transfer mechanisms [5–7].

Elevated CO_2 levels in the broth are known to negatively affect the fermentation process. The inhibitory effect of CO_2 on microbial growth and propagation may not be completely biochemical in nature and could also include physiochemical inhibition due to exposure to high concentrations of dissolved CO_2 (DCO_2) [1,2,4,12–15]. The inhibitory effect of CO_2 is related to the composition of the fermentation broth and has been found to be dependent on the DCO_2 concentration rather than the off-gas CO_2 concentration [1,4,12,15].

Many efforts have been endeavored to relate CO_2 evolution, measured in the off-gas stream, to yeast performance during ethanol fermentation [9,10,12–14]. The very premise of these investigations is that off-gas CO_2 directly affects yeast performance; however, inhibitory effects are attributed to DCO_2 in the fermentation broth and not CO_2 in the fermenter head space [2,16]. Very few authors [3,6,8,12,16,17] have attempted to directly measure dissolved or aqueous phase CO_2 concentrations; most have calculated the DCO_2 concentration on assumption of equilibrium between aqueous and gaseous phase CO_2 using several empirical and semi-empirical models [6,7,16,17,24]. However, the discrepancy related to the absence of any off-gas CO_2 during the initial hours of fermentation (0–8 h) [9,10] is attributed to CO_2 dissolution in the aqueous fermentation broth and the absence of equilibrium between CO_2 in dissolved and gaseous forms [6,15,17]. Despite these shortcomings authors have persisted on measuring off-gas CO_2 and developing various models to determine the corresponding DCO_2 concentration.

We postulate this discrepancy will not be evident in DCO_2 measurements, and in this article report the characteristics of DCO_2 profiles under four different glucose levels during ethanol fermentation. An effort has also been made to elucidate the dissolved CO_2 data obtained from the experiments in terms of their physical, chemical and mathematical significance. Unlike previous

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Nomenclature

$CER(t)$	carbon dioxide evolution or production rate (mol L^{-1} h)
$[CO_2]$	total carbon dioxide concentration in the system (mol L^{-1})
$[CO_2]^*$	equilibrium carbon dioxide concentration (mol L^{-1})
$[CO_3^{2-}]$	concentration of carbonate ions (mol L^{-1})
$[DCO_2]$	total dissolved carbon dioxide concentration (mol L^{-1})
$[HCO_3^-]$	concentration of bicarbonate ions (mol L^{-1})
H^{CO_2}	Henry's law constant for CO_2 (L atm mol^{-1})
$[H^+]$	concentration of hydrogen ions (mol L^{-1})
K_1, K_2	equilibrium constant (mol L^{-1})
$K_L^{CO_2}a$	volumetric mass transfer coefficient for CO_2 (h^{-1})
k_b	reaction rate constant for forward reaction (h^{-1})
k_{-b}	reaction rate constant for reverse reaction (L mol^{-1} h^{-1})
p_{CO_2}	partial pressure of off-gas CO_2 (atm)
X	biomass in terms of viable cells (g viable cell dry weight)
Y_{CO_2}	biomass yield coefficient w.r.t. CO_2 (g CO_2/g viable cell dry weight)
μ	specific growth rate of *S. cerevisiae* (h^{-1})
$\hat{\mu}$	maximum specific growth rate of *S. cerevisiae* (h^{-1})

endeavors in this area, the proposed mathematical models are based on DCO_2 concentration rather than off-gas CO_2.

2. Materials and methods

2.1. Strain and growth media

The active dry yeast (Ethanol Red™) was obtained from Lesaffre Yeast Corp. (Milwaukee, MI, USA). The dry yeast was rehydrated with 50 mL sterilized water, and cultured in YPD agar (10 g/L yeast extract, 10 g/L peptone, 20 g/L dextrose, and 20 g/L agar). Two subculture steps were performed to purify yeast strains for later use. Yeast extract was obtained from HiMedia Laboratories (Mumbai, India). All other chemicals were of reagent grade or higher purity. The growth medium consisted of yeast extract (1%, w/v), sodium glutamate (0.1%, w/v), $MgSO_4{\cdot}7H_2O$ (2 mM), KH_2PO_4 (3.67 mM), urea (16 mM), $(NH_4)_2SO_4$ (1 mM), and mineral salts, including 24 μM H_3BO_3, 1.5 μM Na_2MoO_4, 20 μM $MnSO_4{\cdot}H_2O$, 10 μM $CuSO_4$, 1.8 μM KI, 100 μM $FeCl_3{\cdot}6H_2O$, 82 μM $CaCl_2{\cdot}2H_2O$, and 1000 μM $ZnSO_4{\cdot}7H_2O$. The growth medium was steam sterilized at 121 °C for 15 min.

2.2. Fermentation

Fermentation was carried out in jar fermenters (Model: Omni Culture, New York, NY, USA) with a 1 L working volume at 33 °C. The agitation rate was kept at 150 rpm for all runs. The initial glucose concentrations were 150 ± 0.0, 200 ± 0.21, 250 ± 0.12, and 300 ± 0.28 g/L. The yeast culture was cultivated to its mid-exponential phase in a shake flask, and then inoculated in the fermenter. The shake flask cultures were cultivated in a 100 mL broth (20 g glucose/L, 1% (w/v) yeast extract, 0.2% (w/v) $MgSO_4$ and 0.5% (w/v) urea) at 32 °C and 120 rpm for 18 h prior to inoculation. The inoculum level was 5% of the working volume, and the pitching rate was adjusted to ca. 10^7 viable yeast cells per mL for all experiments. The cell viability was determined using the methylene violet staining technique [25]. All experiments were performed in triplicate.

2.3. Dissolved CO_2 measurement

Each fermenter was equipped with an autoclavable CO_2 electrode (InPro® 5000, Mettler-Toledo, Bedford, MA, USA). The voltage signal was converted to CO_2 concentration by a M400 controller (Mettler-Toledo, Bedford, MA, USA) and acquired using LabView (Version 8.5, National Instrument, Austin, TX, USA). The electrode was calibrated using a 1-point calibration procedure provided by the manufacturer. The measurement of dissolved CO_2 by InPro® 5000 electrode is based on the Severinghaus potentiometric principle, which has been implemented to build custom dissolved CO_2 sensors [15,18–20].

2.4. Determination of ethanol toxic concentrations

The biomass and ethanol concentrations were smoothed by three-parameter logistic growth models as described in Section 2 of [21]. A plot of biomass production rate (first-order derivative of biomass concentration against time) vs. ethanol concentration was created (plot not shown). The ethanol concentration corresponding to where the biomass production rate approaches to zero was considered to be the ethanol toxic concentration that was determined to be 85 g/L.

2.5. Sample analysis

A 5-mL fermentation broth aliquot was withdrawn every 6 h. The broth was quantified by high performance liquid chromatography (HPLC) (Model: HP 1100 series, Agilent Technologies, Mississauga, ON, Canada) employing a refractive index (RI) detector (Model: 1200 series from Agilent Technologies). An ion exclusion column (Model: ORH-801, Transgenomic, Inc., Omaha, NE, USA) was used to separate the metabolites. The mobile phase consisted of 40 mM H_2SO_4 (HPLC grade) at a flow rate of 0.25 mL/min. Column and RI detector temperatures were maintained at 65 and 35 °C, respectively. Biomass dry weight was obtained by substituting the optical density values read at 600 nm (Klett™ Colorimeter, Belart, NJ, USA) into a pre-determined calibration curve that relates biomass concentration to optical density measurements.

2.6. Construction of DCO_2 mass balance equation

The DCO_2 profile measured in the current series of experiments can be illustrated in terms of a DCO_2 mass balance as shown in Eq. (1). Eq. (1) describes the behavior of CO_2 in the fermentation broth in terms of physiochemical and biological processes that occur within the system. The CO_2 evolution rate, the first term on the right hand side of Eq. (1), refers to the rate of CO_2 production by *Saccharomyces cerevisiae*. The CO_2 desorption rate, the second term on the right hand side of Eq. (1), refers to the removal or liberation of DCO_2 from the aqueous to the gas phase while the rate of conversion of DCO_2 to HCO_3^- ions is defined by Eqs. (3) and (4).

$$\left\{\begin{array}{l}\text{Rate of accumulation}\\ \text{of } CO_2 \text{ in aqueous}\\ \text{phase}\end{array}\right\} = \left\{\begin{array}{l}CO_2 \text{ evolution rate}\\ \text{by yeast}\end{array}\right\} - \left\{\begin{array}{l}CO_2 \text{ desorption rate}\\ \text{by physiochemical}\\ \text{process}\end{array}\right\} - \left\{\begin{array}{l}\text{Rate of conversion}\\ \text{of } HCO_3^- \text{ from } CO_2\end{array}\right\} \quad (1)$$