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# Biological conversion of carbon monoxide to ethanol: Effect of pH, gas pressure, reducing agent and yeast extract

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ABSTRACT

A two-level full factorial design was carried out in order to investigate the effect of four factors on the bioconversion of carbon monoxide to ethanol and acetic acid by *Clostridium autoethanogenum*: initial pH (4.75–5.75), initial total pressure (0.8–1.6 bar), cysteine–HCl·$H_2O$ concentration (0.5–1.2 g/L) and yeast extract concentration (0.6–1.6 g/L). The maximum ethanol production was enhanced up to 200% when lowering the pH and amount yeast extract from 5.75 to 4.75 g/L and 1.6 to 0.6 g/L, respectively. The regression coefficient, regression model and analysis of variance (ANOVA) were obtained using MINITAB 16 software for ethanol, acetic acid and biomass. For ethanol, it was observed that all the main effects and the interaction effects were found statistically significant ($p < 0.05$). The comparison between the experimental and the predicted values was found to be very satisfactory, indicating the suitability of the predicted model.

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## 1. Introduction

Biological conversion of waste gases containing carbon monoxide (CO) using acetogens offers a possibility through which waste can be efficiently utilized for generating valuable fuels like ethanol, butanol and hydrogen (Mohammadi et al., 2011; Munasinghe and Khanal, 2010). Different bioreactors can be used for (waste) gas treatment or bioconversion (Abubackar et al., 2011a; Kennes et al., 2009). However, one major bottleneck for the commercialization of this technique is the poor aqueous solubility of carbon monoxide gas. Hence, for systems containing CO as sole substrate, the bioconversion process is limited by the CO gas–liquid mass transfer at high cell concentration. Besides, the process is kinetically limited when either the cell concentration or the CO consumption rate is too low (Abubackar et al., 2011a). These rate-limiting conditions would decrease the process yield and CO–bioconversion process and are often encountered at some point in the bioconversion.

Homoacetogens able to produce ethanol from carbon monoxide include *Clostridium ljungdahlii*, *Clostridium carboxidivorans* P7$^T$, *Clostridium ragsdalei*, *Alkalibaculum bacchi*, *C. autoethanogenum*, *Clostridium drakei*, and *Butyribacterium methylotrophicum*, among others (Liu et al., 2012 ; Mohammadi et al., 2011, 2012). These unicarbonotrophic bacteria follow the acetyl-CoA biochemical pathway or Wood-Ljungdahl pathway for cell growth and product formation (Abubackar et al., 2011a). Apart from ethanol, acetic acid is one of the prominent metabolites found during CO conversion using these microorganisms. In most of the previous studies, low ethanol to acetic acid ratios were generally obtained. However, by optimizing the medium composition and operating conditions, this ratio can be increased (Kundiyana et al., 2011a,b). In the present research, a microcosm study was performed using *C. autoethanogenum* as the biocatalyst.

*C. autoethanogenum* is a strictly anaerobic gram positive rod shaped (0.5 × 3.2 μm) bacterium, originally isolated from rabbit faeces using CO as the sole carbon and energy source. (Abrini et al., 1994). In one study, the authors used Plackett–Burman design to screen significant ethanol enhancing factors from the defined medium developed for *C. carboxidivorans*. Optimal levels of these significant factors were evaluated by central composite design (CCD) using a response surface methodology (RSM) and an artificial neural network-genetic algorithm (ANN-GA). It was concluded that an optimal medium containing (g/L) NaCl 1.0, $KH_2PO_4$ 0.1, $CaCl_2$ 0.02, yeast extract 0.15, $MgSO_4$ 0.116 and $NH_4Cl$ 1.694, at pH 4.74 could yield an ethanol concentration of around 0.25 g/L (Guo et al., 2010). Another research reported a concentration of 0.06–0.07 g/L with a 1:13 ethanol to acetate ratio in liquid-batch continuous syngas fermentation using a xylose adapted *C. autoethanogenum* culture (Cotter et al., 2009). These studies reveal the importance of medium composition in increasing the overall ethanol production. Hence, the different operating conditions still have to be optimized in order to enhance ethanol production and save on operating costs.

In the present research, *C. autoethanogenum* was used to convert bottled carbon monoxide gas into a valuable fuel product such as

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