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Enrichment and optimization of anaerobic bacterial mixed culture for conversion of syngas to ethanol

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HIGHLIGHTS

- Anaerobic bacterial mixed culture enriched for conversion of syngas to ethanol.
- Operational parameters optimized for enhancing ethanol production from syngas.
- Semi-continuous fermentation study done for getting increased ethanol production.
- Up-scaling studies done for further enhancing ethanol production from syngas.

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ABSTRACT

The main aim of the present study was to enrich anaerobic mixed bacterial culture capable of producing ethanol from synthesis gas fermentation. Screening of thirteen anaerobic strains together with enrichment protocol helped to develop an efficient mixed culture capable of utilizing syngas for ethanol production. Physiological and operational parameters were optimized for enhanced ethanol production. The optimized value of operational parameters i.e. initial media pH, incubation temperature, initial syngas pressure, and agitation speed were 6.0 ± 0.1, 37 °C, 2 kg cm^{-2} and 100 rpm respectively. Under these conditions ethanol and acetic acid production by the selected mixed culture were 1.54 g L^{-1} and 0.8 g L^{-1} respectively. Furthermore, up-scaling studies in semi-continuous fermentation mode further enhanced ethanol and acetic acid production up to 2.2 g L^{-1} and 0.9 g L^{-1} respectively. Mixed culture TERI SA1 was efficient for ethanol production by syngas fermentation.

1. Introduction

Synthesis (syngas) gas, primarily a mixture of CO, CO_2 and H_2 is a major feedstock in production of many fuels and chemicals. It can be produced from gasification of several materials such as coal, wood, municipal solid waste and lignocellulosic biomass (Phillips et al., 1993). The essential syngas components CO, CO_2 and H_2 can be biologically converted into ethanol and other value added compounds such as acetic acid, 2-butanol, n-propanol and polyhydroxyalkanoate (PHA) (Kundiyana et al., 2010). Catalytic processes are also used to convert syngas components into a variety of fuels and chemicals such as hydrogen, methane, methanol, ethanol, and acetic acid (Klasson et al., 1992). Biological processes, although relatively slower than chemical reaction have several advantages over catalytic process such as elimination of expensive metal catalyst, higher specificity of biocatalyst, lower energy costs, greater resistance to catalyst poisoning and independence of H_2/CO ratio (Wolfrum and Watt, 2002). The biological reaction occurs under ambient condition of pH, temperature and pressure with the formation of specific products. However, direct production of fuels and chemicals from gasification technology is economically unfavorable and requires very large infrastructure as about 60% of the total investment cost in modern methanol plants is accounted for syngas generation (Vannby and Winter Madsen, 1992). Therefore, the economical advantage of biological processes through development of suitable biocatalyst to ferment gaseous substrate to valuable products can be considered. Previous study indicates that FT process has a relative overall energy efficiency of 45%, while gas

Abbreviations: PHA, polyhydroxyalkanoate; PBM, Pfennig's basal media; TCD, thermal conductivity detector; FID, flame ionization detector; OD, optical density; CO, carbon monoxide; H_2, hydrogen; rs, resparged; AA, acetic acid; EtOH, ethanol; SD, Sludge; CD, cow dung; CF, chicken faeces; VFA, volatile fatty acids.

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fermentation has an overall energy efficiency of 57%, in terms of energy in feedstock converted to final product (Griffin and Schultz, 2012).

Microorganisms act as biocatalysts to convert syngas into chemicals and fuels. Anaerobic bacteria such as *Clostridium ljungdahlii*, *Clostridium ragsdalei*, *Clostridium carboxidivorans*, *Clostridium aceticum*, *Acetobacterium woodii* and *Clostridium thermoaceticum* convert biomass generated syngas composed of CO, CO_2 and H_2 to ethanol and acetic acid (Kundiyana et al., 2010). Bioethanol production through acetogenic fermentation of the gaseous substrates (CO, CO_2 and H_2) follows the Wood-ljungdahl and acetyl-CoA pathways. Syngas fermentation for ethanol production based on the acetyl-CoA metabolic pathway is an emerging area and the process require significant research interventions. Several research groups have explored the use of anaerobic bacteria to convert syngas to ethanol (Gaddy and Clausen, 1992; Younesi et al., 2005). However, these studies have yet to define a methodology for generating high ethanol production levels with a stable culture. The selection of appropriate microbes for efficient syngas fermentation is often a challenging task. Therefore, the isolation and engineering of new microbial species, which are more productive and robust, need to be employed. Results from published literature show that temperature, pH, reducing agents, redox potential, agitation speed, gas partial pressure, gas compositions and media components have significant effects on cell growth and ethanol production (Hurst and Lewis, 2010). Optimization of these parameters is critical for assessing the potential sustainability of ethanol from syngas.

In the recent years, a general understanding regarding cell growth and metabolism leading to higher conversion of syngas to liquid fuel has been made. Syngas fermentation using *Clostridium P7* showed that increase in CO partial pressure from 0.35 to 2.0 atm. resulted in 440% improvement in cell growth and ethanol production shifted from non-growth associated to growth associated phase (Hurst and Lewis, 2010). Studies on agitation speed showed 50% decrease in ethanol production when agitation speed was increased to 250 rpm from 150 rpm (Ramachandriya, 2006). Similar studies on bioreactor design and up-scaling parameters showed six fold increase in ethanol production when syngas fermenting strain *Clostridium P11* was scaled up from 7.5 L to 100 L pilot scale fermentor (Kundiyana et al., 2010). Furthermore, studies on the aspects of process optimization have also enhanced the conversion rates and ethanol production (Kundiyana et al., 2011).

Based on the points highlighted above, the present study was aimed at developing anaerobic mixed bacterial culture that have the capability to utilize syngas for ethanol production. However there is a considerable number of studies on syngas fermentation using mesophilic pure cultures (Tanner, 2005; Maddipati et al., 2011). Limited studies using mixed-cultures have been reported, although mixed culture fermentation is more suited for industrial applications, when compared to pure culture fermentation. Some of the advantages are: (i) no need for highly sterile cultivation, (ii) presence of high microbial diversity, which offers increased adaptation capacity, (iii) possibility of mixed substrates co-fermentation, and (iv) higher capacity for continuous processing. The mixed culture presents an opportunity for higher alcohols production from syngas. Semi-continuous fermentations in a 3 L fermentor with the mixed culture and CSL medium resulted in a twofold more total alcohol production than in the YE medium. The synergy between strain CP15 and *Clostridium propionicum* in the mixed culture in bottle fermentations resulted in 50% higher efficiency in converting propionic acid, butyric acid and hexanoic acid to their respective alcohol (Liu et al., 2013). Further, different operational parameters such as pH, temperature, syngas pressure and agitation speed were optimized for enhanced ethanol production from syngas. In addition, semi-continuous and scale-up fermentation studies from 130 mL to 1 L volumes were also carried out to enhance the ethanol production.

2. Methods

2.1. Source of inoculum and fermentation medium

Micro-organisms used for the present study were taken from TERI's culture collection. TERI is a referral centre and culture repository for bacterial strains in India (Sarma et al., 2004; Agrawal et al., 2010; Kaur et al., 2009; Singh et al., 2014). An additional approach of enrichment was also employed using sludge (SD), cow dung (CD) and chicken faeces (CF) samples collected from Gwal pahari (Gurgaon, Haryana, 28°28′N 77°1.9′E) India. Pfennig's basal media (PBM) medium containing (per liter) 50 mL mineral stock solution, 10 mL trace metal stock solution, 10 mL vitamin stock solution, 1 g yeast extract, 1 mL resazurin (0.1%) and 20 mL L-cysteine HCl (2.5%) was used for the enrichment of microbial strains. The composition of the mineral, vitamin and trace metal stock solutions has been previously reported (Gaddy and Clausen, 1992). Unless specified, all the experiments were performed in 130 mL Wheaton serum bottles containing 40 mL of the liquid medium. Anaerobic condition during media preparation was maintained as described by (Singh et al., 2014). The bottles were sealed with butyl rubber stopper and aluminum cap and then sterilized at 121 °C and 15 psi for 15 min. Vitamin solution was added after sterilization. The initial pH of the medium was adjusted at 6.0 ± 0.1 with 2 N KOH and 2 N HCl solutions.

2.2. Screening and enrichment of syngas utilizing microbial mixed culture for ethanol production

In the present study initially 13 microbial cultures were collected from TERI's culture collection (Sarma et al., 2004; Agrawal et al., 2010; Kaur et al., 2009; Singh et al., 2014) and screened for syngas fermentation to ethanol. An additional approach of enrichment was also designed. Sludge, cow dung and chicken faeces samples were used for the enrichment of syngas utilizing microbial mixed culture. All samples were subjected to multiple enrichment cycles by adding (10% w/v) sample inoculum into 125 mL of Wheaton serum bottle with 40 mL of freshly prepared medium. The culture bottles were sparged and headspace pressurized up to 1 kg cm^{-2} with commercial syngas (CO 20%, CO_2 15%, H_2 20%, CH_4 3% balance N_2). Incubation was done at 37 °C in a rotary shaker at 150 rpm. After 5 days (10% v/v) enriched culture was subsequently transferred into fresh medium and this process was repeated for 6 cycles. During each sub-culturing cycle the culture bottles were analyzed for syngas utilization, Volatile fatty acids (VFA) and solvent production (as described in Section 2.6). The enrichment cycles were done in duplicates.

2.3. Optimization of operational parameters for enhanced ethanol production by the selected mixed culture

Based on enrichment results, selected syngas fermenting mixed culture was taken up for further study. Different operational parameters were investigated for enhanced ethanol production. To investigate the effect of temperature on growth and ethanol production by the selected mixed culture, the experimental set was incubated at temperatures 30 °C, 37 °C and 50 °C at pH 6.0, agitation speed 150 rpm and syngas pressure in the reactor bottle headspace of 1 kg cm^{-2}. Effect of initial medium pH on ethanol production by selected mixed culture TERI SA1 was studied with the pH 5.0, 6.0, 7.0 and 8.0. The pH was adjusted with 2 N HCl