**Screening of medium and process optimization for high cell density production of BL21 (DE3) cells containing clostridial cellulolytic chimeric (CtGH1-CtGH5-F194A) enzyme**

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**Abstract**

Petroleum-based fuels cause enormous pollution in the environment. Additionally, the energy requirement in the world is increasing exponentially and crude oil reserves are depleting. This may lead to “no fuel” left for future generations. Therefore, it has become necessary to find an alternative to non-renewable sources of energy. Biofuels can be one of the best alternatives for fossil fuels. Lignocellulosic biomass comprises structural carbohydrates with cellulose, hemicellulose and lignin. Most of the lignocellulosic biomass is being burnt every year, which can be used for bioethanol (Biofuel) production. Three cellulolytic enzymes (endoglucanase, cellobiohydrolase and β-glucosidase) act in synergistic manner to hydrolyse cellulose into monomer glucose unit. To make this process cost effective, Nath et al., 2019 constructed a recombinant plasmid containing gene encoding chimeric enzyme (*Ct*GH1-L1-*Ct*GH5-F194A) within pET28a (+) vector and expressed it in BL21 (DE3) *E. coli* cells. Both the enzymes of chimera were isolated from thermophilic bacterium, *Clostridium thermocellum* and reported bifunctional activity of β-glucosidase and endoglucanase which can help in reducing the enzyme production cost in biorefinery. The current study is focused on the process optimization for high cell density production of BL21 (DE3) cells containing clostridial chimeric protein. Better protein expression was observed with 0.25 mM IPTG concentration at 24 °C as compared to 0.5 mM and 1 mM IPTG concentration as well as 16 °C and 24 °C temperature. The preliminary study on the selection of medium from different commercial media like Luria Bertani (LB), Terrific broth (TB), Autoinduction medium (AIM), Super optimal broth (SOB) and tryptone yeast extract (TYE) each in 200 mL. Maximum cell optical density (at OD600) was observed in TB and LB media i.e., 2.9 and 2.5 with maximum specific growth rate (µmax) of 0.23 h-1 and 0.22 h-1, respectively, after 24 h of culture growth. However, Autoinduction (2.1 with 0.22 h-1), SOB (1.9 with 0.15 h-1) and TYE (0.8 with 0.10 h-1) media did not show significant cell density after 24h. The estimated dry cell weight (DCW) of 23h grown culture for each medium was, TB: 1.7g/L, LB: 1.2g/L, SOB: 1.1g/L AIM: 0.85g/L and TYE: 0.6g/L (Table 1). Total crude protein was estimated using Lowry’s method and enzyme activity for both the modules were estimated by Nelson Somogyi and GOD POD analysis method as shown in Table 1. Further these results will be compared with M9 minimal media and process optimization for high cell density culturing will be done

**Keywords:** Bioethanol, Biorefinery, Chimera, Cellulolytic enzymes

**Table 1 Production characteristics of *E. coli* expressing cellulolytic chimera (*Ct*GH1-L1-*Ct*GH5-F194A) grown on various media**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Medium** | **Total Cell biomass (gDCW/L)** | **crude protein concentration (mg/mL)** | **Endoglucanase Activity (U/mL)** | **Β-glucosidase Activity (U/mL)** |
| **TB** | **1.7** | **3.45 ± 0.43** | **0.11** | **1.05** |
| **LB** | **1.2** | **1.53 ± 0.19** | **0.10** | **0.35** |
| **SOB** | **1.1** | **2.53 ± 0.10** | **0.12** | **0.23** |
| **AIM** | **0.85** | **1.43 ± 0.10** | **-** | **-** |
| **TYE** | **0.6** | **1.26 ± 0.10** | **-** | **-** |