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Establishment of High-Temperature Simultaneous Saccharification and Fermentation Process by Co-Culturing of Thermally Adapted Thermosensitive Saccharomyces Cerevisiae and Bacillus amyloliquefaciens

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Abstract : Recent global warming created by various pollutants prompted us to find new energy sources instead of fossil fuels. Fossil fuels are one of the key factors to emit various toxic gases in this planet. To solve this problem, along with the scarcity of the worldwide energy crisis, scientists are looking for various alternative options to mitigate the necessity of required future fuels. In this context, bioethanol can be one of the most suitable alternative energy sources. Bioethanol is a renewable, environment-friendly and carbon-neutral sustainable energy. In our previous study, we identified several bioethanol-producing microbes from the natural fermented sources of Bangladesh. Among them, the strain 4C encoded Saccharomyces cerevisiae produced maximum bioethanol when the fermentation temperature was 25˚C. In this study, we have established high-temperature simultaneous saccharification and fermentation process (HTSSF) by co-culturing of thermally adapted thermosensitive 4C as a fermenting agent and Bacillus amyloliquefaciens (C7), as a saccharifying agent under various physiological conditions or treatments. Conventional methods were applied for cell culture, media preparation and other experimental purposes. High-temperature adaptation of strain 4C was made from 30-42°C, using either YPD or YPS media. In brief, for thermal adaptation, the temperature was periodically increased by 2°C, 1°C and 0.5°C when medium growth temperatures were 30-36°C, 36-40°C, and 40-42°C, respectively, where applicable. Amylase activity and bioethanol content were measured by DNS (3, 5-dinitrosalicylic acid) and solvent extraction and dichromate oxidation method, respectively. Among the various growth parameters like temperatures (30˚C, 37˚C and 42˚C), pHs (5.0, 6.0 and 7.0), carbon sources (5.0-10.0%) and ethanol stress tolerance (0.0-12.0%) etc. were tested, maximum Amylase activity (4.0 IU/ml/min) was recorded for Bacillus amyloliquefaciens (C7) at 42˚C, pH 6.0 and 10% starch. On the other hand, 4.10% bioethanol content was recorded when the thermally adapted strain 4C was co-cultured with C7 at 37°C, pH 6.0 and 10.0% starch for 72 hours at HTSSF process. On the other hand, thermally non-adapted strains gave only 0.5-2.0% bioethanol content under the same physiological conditions. The thermally adapted strain 4C and strain C7, both can tolerate ethanol stress up to 12%. Altogether, a comparative study revealed that our established HTSSF process may be suitable for pilot scale and subsequently at industrial level bioethanol production.

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