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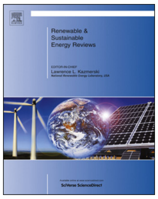

Bioprospecting thermophilic/thermotolerant microbes for production of lignocellulosic ethanol: A future perspective

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ABSTRACT

The progressive depletion of non-renewable energy sources worldwide, together with the fact that their overexploitation has resulted in environmental deterioration and public health problems, has led to consider alternative sources of energy. Lignocellulose-based bioethanol is a leading option among alternatives to petroleum-derived transportation fuels due to its potential sustainability. The production of ethanol through microbial fermentations has generated considerable research interests. Several thermophilic/thermotolerant ethanologenic species i.e. *Clostridium thermocellum*, *C. thermohydrosulfuricum*, *C. thermosaccharolyticum*, *Caldicellulosiruptor* sp., *Thermotoga* sp., *Thermoanaerobium brockii*, *Thermoanaerobacter ethanolicus*, *T. thermo-hydrosulfuricus*, *T. mathranii*, etc., have been isolated and identified as the potential lignocellulosic ethanol producers. Use of lignocellulolytic organisms alone at high temperatures could potentially reduce the cellulase requirement. Moreover, such cultures facilitate the ethanol production at high temperature and offer the possibility of in-situ ethanol recovery. However, more research on the metabolic pathways, regulation of end-product formation and construction of genetically engineered thermophilic/thermotolerant microorganisms with high tolerance to ethanol is required for optimal utilization of such microbes in industrial fermentations. Therefore, the present review has been focused on thermophilic/thermotolerant microbes for the production of ethanol, especially on their catabolic pathways, end-product formation and their future perspectives for industrial applications.

Contents

Abbreviations: ABE, acetone–butanol–ethanol; ADH, alcohol dehydrogenase; AFEX, ammonia fiber explosion; ALDH, acetaldehyde dehydrogenase; ARAA, arabinose isomerase; ARAB, ribulokinase; ARAD, ribulosephosphate-4-epimerase; CBP, consolidated bioprocessing; CF, co-fermentation; CNG, compressed natural gas; DDGS, dried distillers grains and soluble; EMB, embden Meyerhof Parnas; EPFB, empty palm fruit bunch; ETBE, ethyl tertiary butyl ether; FNOR, ferredoxin/NAD(P)H oxidoreductase; GK, glucokinase; LCB, lignocellulosic biomass; LGE, litres of gasoline equivalent; MESP, minimum ethanol selling price; MH, microbial hydrolysis; PDC, pyruvate decarboxylase; PDH, pyruvate dehydrogenase; Pebax/POSS, polyether-block-amide/polyhedral oligosilsesquioxane; PFL, pyruvate formate lyase; PFOR, pyruvate ferredoxin oxidoreductase; PPP, pentose phosphate pathway; SeSF, semi-Solid fermentation; SHF, separate hydrolysis and fermentation; SmF, sunmerged fermentation; SoSF, solid state fermentation; SSCF, simultaneous saccharification and co-fermentation; SSF, simultaneous saccharification and fermentation; TAL, transaldolase; t_d, doubling time; TKL, transketolase; TPP, thiamine pyrophosphate; XD, xylose dehydrogenase; XDH, xylitol dehydrogenase; XI, xylose isomerase; XK, xylilokinase; XR, xylose reductase; XYLB, xylulokinase

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1. Introduction

The global rise in energy consumption, predicted increase in energy demands in the near future, the depletion of low extraction cost fossil fuel reserves, and climate change are the driving forces to search for renewable and eco-friendly energy resources [1]. Many policies have been designed to encourage the production and use of renewable bioenergy keeping their impact on the environment into consideration [2]. Keeping in view the current strategies in the field of bioenergy, it is also expected that by 2050, bioenergy will contribute to 30% of the world's energy demand [3]. An alternative fuel must be technically feasible, economically competitive, environmentally acceptable, and readily available throughout the year. Numerous potential alternative fuels have been proposed, including bioethanol, biobutanol, biodiesel, methanol, hydrogen, CNG, biogas, Fischer–Tropsch fuel, electricity, and solar fuels [4]. However, 3% of the transport fuels requirement on roads and in aviation and marine sectors is provided by the liquid biofuels [5].

Among liquid biofuels, bioethanol has been proven to be an alternative transportation fuel with the largest potential to replace fossil derived fuels, responsible for a significant fraction of greenhouse gas emissions. It also improves the fuel combustion in vehicles and reduces the emission of carcinogens into the environment [6,7]. According to a report of Alternative Fuels and Data Center, USA is one of the largest producer of bioethanol from 2007 to 2013 and Brazil is second in the list as shown in Fig. 1 [8]. However, the latter is the leading exporter of bioethanol to United States and EU-27 countries with an export of 325 and 49 million liters, respectively [9].

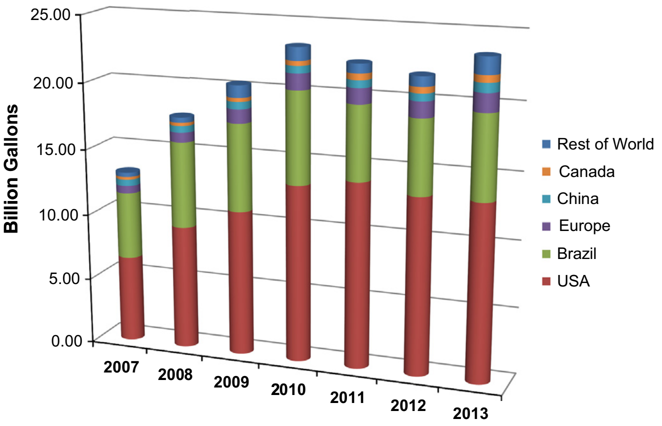

Fig. 1. World fuel ethanol production (billion gallons).

Bioethanol has been used as a modern biofuel, gasoline improver, gasoline subsistent, in the form of ETBE as octane enhancers and as bioethanol–diesel blends [10]. Engine modification is not required in case of lower level 5% blend which covers under vehicle warranties. However, higher level blending of bioethanol, for example, E85 requires engine modification [11]. Recently, some countries have exercised mandatory bioethanol–gasoline blend under biofuel program from E2 to E20 [12].

Current bioethanol research focuses on lignocellulosic feedstocks due to their abundance and renewability; especially in relation to reducing the cost and increasing the efficiency of the key steps in the lignocelluloses-to-bioethanol process (e.g. lignocellulosic pre-treatment, enzymatic hydrolysis and fermentation) [13–16]. The main advantage of using LCB for bioethanol production is to limit the direct food versus fuel competition associated with first generation biofuels [7,17].

Apart from using LCB, another major shift in bioethanol production is towards the use of thermophilic/thermotolerant microorganisms. These microorganisms possess many industrially important properties which give them better score over the mesophillic microorganisms. They have the potential to use wide range of LCB with acid and salt tolerance properties. High temperature favours the continuous removal and recovery of ethanol from broth under reduced pressure, thereby minimizing the risk of toxicity level of ethanol for the culture and reducing the cost required for cooling which aids in the process economics [18]. Besides this, there is high rate of diffusion/mixing of nutrients at high temperature which further increases the productivity and growth rate of the culture and reduces the risk of contamination. Moreover, there is lesser cell mass yield, so more of the substrate is converted into the end product [19]. Fermentation of the pentose sugars in addition to the hexose sugars by the thermophilic/thermotolerant microorganisms further increase their value in the biofuel industry as pentose sugars are present in good amount in the LCB [20,21].

The high cost of lignocellulolytic enzymes is a major setback for the biofuel industry [22]. However, saccharification at higher temperatures has many advantages. Major saccharifying enzymes (cellulases and hemicellulases) produced by thermophilic/thermotolerant microorganisms are highly thermoactive and thermostable in the presence of high concentrations of organic solvents, detergents and alcohols and thus, negate the use of costly commercial enzymes. Thermozymes have more flexibility with respect to process configurations and high specific activity which decrease the total amount of enzyme required for hydrolysis [4]. Decrease in viscosity at high temperature increases the diffusion coefficients of the substrates for efficient enzymatic hydrolysis [23,24]. All these factors improve the efficiency of the bioprocess