Biochemistry and Biophysics Reports 10 (2017) 52–61

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Yeasts in sustainable bioethanol production: A review

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ARTICLE INFO

Keywords:
Yeasts
Fermentation
Bioethanol
Immobilization

ABSTRACT

Bioethanol has been identified as the mostly used biofuel worldwide since it significantly contributes to the reduction of crude oil consumption and environmental pollution. It can be produced from various types of feedstocks such as sucrose, starch, lignocellulosic and algal biomass through fermentation process by microorganisms. Compared to other types of microoganisms, yeasts especially *Saccharomyces cerevisiae* is the common microbes employed in ethanol production due to its high ethanol productivity, high ethanol tolerance and ability of fermenting wide range of sugars. However, there are some challenges in yeast fermentation which inhibit ethanol production such as high temperature, high ethanol concentration and the ability to ferment pentose sugars. Various types of yeast strains have been used in fermentation for ethanol production including hybrid, recombinant and wild-type yeasts. Yeasts can directly ferment simple sugars into ethanol while other type of feedstocks must be converted to fermentable sugars before it can be fermented to ethanol. The common processes involves in ethanol production are pretreatment, hydrolysis and fermentation. Production of bioethanol during fermentation depends on several factors such as temperature, sugar concentration, pH, fermentation time, agitation rate, and inoculum size. The efficiency and productivity of ethanol can be enhanced by immobilizing the yeast cells. This review highlights the different types of yeast strains, fermentation process, factors affecting bioethanol production and immobilization of yeasts for better bioethanol production.

1. Introduction

The improvement of living standard urges the hunt for sustainable energy in order to meet energy consumption across the world [1]. On the other hand, the use of fossil fuels as the main energy resources caused the arising of worldwide problems such as environmental pollution and global warming [2,3]. These led to the finding of environmentally friendly, renewable and sustainable energy by government, industrial and energy sector [4,5]. Among renewable energies, priority was given to liquid biofuels as it represents about 40% of the total energy consumption in the world [6]. The use of liquid biofuels contributes to the reduction of greenhouse gas emissions, creation of job opportunities, regional development and supply security [5,7].

Bioethanol is known as the most widely used biofuel in transportation sector and have a long history as alternative fuels. In 1984, Germany and France started to use bioethanol as a fuel in internal combustion engines (ICEs) [8]. Utilization of bioethanol by Brazil was initiated since 1925. In Europe and United States, bioethanol was widely used until the early 1900s. After World War II, the use of bioethanol was neglected due to its expensive production cost compared to petroleum fuel until the oil crisis in the 1970s [5]. The interest in using bioethanol has been increasing since the 1980s and it has been considered as an alternative fuel in many countries. Global ethanol production increased from 13.12 billions of gallons in 2007 to 25.68 billions of gallons in 2015 with a slight decreased in 2012 and 2013 [9]. United States is the largest ethanol producer with the production of nearly 15 billion gallons in 2015. The production of ethanol by United States and Brazil contribute to 85% world's ethanol production.

Bioethanol is also known as ethyl alcohol or chemically C_2H_5OH or EtOH. It can be used directly as pure ethanol or blended with gasoline to produce "gasohol" [10]. It can be used as a gasoline improver or octane enhancer and in bioethanol-diesel blends to reduce the emission of exhaust gasses [11]. Bioethanol offers several advantages over gasoline such as higher octane number (108), broader flammability limits, higher flame speeds and increased heats of vaporization [12]. In contrast to petroleum fuel, bioethanol is less toxic, readily biodegradable and produces lesser air-borne pollutants [13]. A variety of feedstocks from the first, second and third generation has been used in

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http://dx.doi.org/10.1016/j.bbrep.2017.03.003
Received 4 November 2016; Received in revised form 10 February 2017; Accepted 4 March 2017
Available online 06 March 2017

bioethanol production. The first-generation bioethanol involves feedstocks rich in sucrose (sugar cane, sugar beet, sweet sorghum and fruits) and starch (corn, wheat, rice, potato, cassava, sweet potato and barley). Second-generation bioethanol comes from lignocellulosic biomass such as wood, straw and grasses. Third-generation bioethanol has been derived from algal biomass including microalgae and macroalgae [14].

Microorganisms such as yeasts play an essential role in bioethanol production by fermenting a wide range of sugars to ethanol. They are used in industrial plants due to valuable properties in ethanol yield (> 90.0% theoretical yield), ethanol tolerance (> 40.0 g/L), ethanol productivity (> 1.0 g/L/h), growth in simple, inexpensive media and undiluted fermentation broth with resistance to inhibitors and retard contaminants from growth condition [15]. As the main component in fermentation, yeasts affect the amount of ethanol yield. In this review, the role of yeasts in bioethanol fermentation and its immobilization techniques will be discussed in order to enhance the production of ethanol for the benefits of mankind.

2. Yeasts

Yeasts are defined as ascomycetous or basidiomycetous fungi that are capable of reproducing by budding or fission and form spores which are not enclosed in a fruiting body [16]. They are first classified based on its sexuality (Ascomycotina or Basidiomycotina) or the lack of sexual phase in the life cycle (Deuteromycotina). The lower taxonomic subdivisions (families, subfamilies, genera, species and strain) are determined by its morphological, physiological and genetic characteristics including sexual reproduction [17].

2.1. Yeasts diversity

The number of discovered yeasts has been increasing from year to year. More than 2500 yeast species were published by 2005. It is assumed that only 1% of yeast species is currently known which represents approximately 1500 species. The total numbers of yeast species on earth are expected to reach 150,000 [18]. The diversity of yeast species in particular niches is determined by its capability of utilizing different carbon source and its nutritional selectivity as it exhibits great specialization for habitat [19]. Yeasts can be isolated from the terrestrial, aquatic and aerial environment. Plant is the preferred habitat of yeasts community. A few species are found to have commensalism or parasitic relationships with animals. Extreme environments like low water potential (high sugar or salt concentration) and low temperature may be inhabited by yeasts [20,21]. The natural habitats of yeasts are summarized in Table 1.

There are a broad diversity of yeast cells including its size, shape and colour. Cell sizes of yeasts are influenced by its species and growth condition. The length of some yeast cells are only 2–3 µm while the other species may reach the length of 20–50 µm [19]. Most yeasts have a width in the range of 1–10 µm. Generally, the sizes of brewing strains of *S. cerevisiae* are larger than laboratory strains [22]. Many yeast species including *Saccharomyces* spp. are ellipsoidal or ovoid in shape and have creamy colour colonies [20,21].

2.2. Molecular genetics of yeasts

The production of bioethanol is founded on the ability of yeasts to catabolize six-carbon molecules such as glucose into two carbon components, such as ethanol, without proceeding to the final oxidation product which is CO_2. Crabtree positive yeasts such as *S. cerevisiae* accumulate ethanol in the presence of oxygen, however *Candia albicans* which is a crabtree-negative yeast catabolizes sugars into CO_2 in the presence of oxygen [23]. The presence of six carbon carbohydrates represses the oxidative respiration pathway in Crabtree positive yeasts and energy for growth is generated via glycolysis. Upon depletion of six carbon molecules, the catabolism shifts to oxidation of two carbon molecules into CO2 [24]. This phenomenon is termed at the 'diauxic shift'. The process of bioethanol production via fermentative metabolism and the diauxic shift is dependent upon the enzyme Alcohol Dehydrogenase (EC 1.1.1.1) which is encoded on the *ADH1*locus. ADH1 catalyzes the reduction of acetaldehyde to ethanol during the fermentation of glucose, it can also catalyze the reverse reaction which is the conversion of ethanol into acetaldehyde, albeit with a lower catalystic efficiency [25].

The yeast S. *cerevisiae* contain two genes that encode ADH, *ADH1* is expressed constitutively, while the expression of *ADH2* is induced by the reduction in the intracellular concentration of glucose. The substrate for the enzyme ADH2 is ethanol [26]. The expression of ADH2 gene is governed by transcription factors and genome sequencing and transcriptome analysis has revealed the structure and DNA binding elements of these regulatory proteins [27]. Recent advances in synthetic biology have focused on re-engineering the ADH gene for greater substrate specificity and improvement of catalytic activity as well as engineering the yeast genome with protein coding genes [28] which improve tolerance to ethanol and catalysis of a wide range of carbon sources [29]. Molecular biologists are actively seeking novel genes encoding ADHs using metagenomic approaches, and this had yielded a number of unique variants [30].

2.3. Yeasts in bioethanol production

Since thousands of years ago, yeasts such as *S. cerevisiae* have been used in alcohol production especially in the brewery and wine industries. It keeps the distillation cost low as it gives a high ethanol

Table 1
Natural yeasts habitats [20,21].

Habitat	Description	Yeasts genera
Plants	• The common niche of yeasts is the interface between soluble nutrients of plants (sugars) and the septic world • insects help in spreading yeasts on the phyllosphere	*Ashbya* spp. *Nematospora* spp.
Animals	• Several yeasts are pathogenic toward humans and animals while others are non-pathogenic (can be found in intestinal tract and skin of warm-blooded animals) • Numerous yeasts are commensal to insect which act as vectors for natural distribution of yeasts.	*Candida* spp. *Cyniclomyces* spp. *Pityrosporum* spp.
Soil	• Considered as reservoir for yeasts long-term survival rather than habitat for free growth • Yeasts can be found only in the aerobic soil layers (10–15 cm)	*Lipomyces* spp. *Schwanniomyces* spp.
Water	• Yeasts can be found in both fresh water and seawater • Estuarine regions usually have higher numbers of yeasts compared to seawater	*Rhodotorula* spp. *Debaryomyces* spp.
Atmosphere	• Yeasts are dispersed by air currents from the vegetative layer above soil surfaces • Only a few yeasts may be expected per volume of air	*Cryptococcus* spp. *Rhodotorula* spp. *Sporobolomyces* spp. *Debaryomyces* spp.
Extreme environment	• Some halotolerant yeasts can grow in nearly saturated brine solution • Osmophilic yeasts were discovered in glacier horizons	*Debaryomyces* spp. *Zygosaccharomyces* spp.