Genetic Modifications of *Saccharomyces cerevisiae* for Ethanol Production from Starch Fermentation: A Review

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**Abstract**

There is a huge demand for developing new technologies for alternative energy sources due to the elevated costs of petroleum and its by-products, depletion of nonrenewable fuel sources, and to eliminate the disadvantages of geopolitical location and environmental pollution caused by high levels of carbon dioxide release. Science is striving to meet this demand and as molecular biology techniques have progressed, genetic engineering tools have been presented as promising future solutions in the form of optimizing the fermentation process to increase the ethanol yield from different carbon sources such as starch. As *Saccharomyces cerevisiae* is not naturally able to ferment starch, it can be genetically manipulated and modulated to improve the fuel production from starchy materials and the amount of cost that is required to produce ethanol would be decreased with these manipulations. General modifications in *S. cerevisiae* include specific gene expressions to gain new properties or improve existing pathways. This review aims to elicit the current status of ethanol production thorough alternative techniques from starch using current genetic engineering applications and to give further directions for high-throughput fermentations using genetically modified *S. cerevisiae* strains.

**Keywords:** *Saccharomyces cerevisiae*; Ethanol; Fermentation; Genetic engineering; Starch

**Introduction**

Global energy consumption is rapidly increasing and it causes an elevation of energy cost and contributes to global warming because of the excessive use of petroleum based energy sources; hence the demand for renewable biomass-derived fuels has increased in recent years. Production of such alternative sources, which could also help establish a sustainable and renewable energy supply, has been popularized. Several countries have already used first generation biofuels, including ethanol, as a primary energy sources such that worldwide biofuel production reached 106 billion liters in 2011 and it is estimated to be 155 billion liters by 2020 [1]. Feedstocks rich in sugars are generally used for ethanol production via fermentation processes. Main sugar sources for ethanol production are presented in Figure 1. Although all of fuel ethanol is obtained from corn glucose in US [2] and sucrose in Brazil [3], starchy materials have been accepted as the major renewable biomass resources for ethanol production due to their low cost and abundance [4]. In this line, corn wheat and tubers from starchy crops are used for ethanol production in North America, Europe, and tropical countries [5].

Manipulation and utilization of starchy resources starts with enzymatic hydrolysis followed by fermentation of sugar molecules and subsequently the elimination of ethanol from culture media. Simultaneous Saccharification and Fermentation (SSF), is an alternative method used for bioethanol production from feedstock and it decreases fermentation costs by reducing equipment requirements since both processes occur in one reactor [6]. Another bonus in the SSF system is that the ethanol production rate is higher than the conventional method [7]. SSF has been applied for ethanol production from starch fermentation and remarkable ethanol yields (0.41 liter ethanol per kg of corn) have been obtained [8]. There have been attempts to increase the fermentation efficiency with immobilization of microorganisms in SSF systems [9-11]. Although SSF has advanced in the field, several points should be optimized to reduce the total cost, provide highly efficient utilization of starch, and maximize ethanol yield. These steps include: maintaining the optimal pH for the growth of fermentative microorganism, sterility, continuous substrate supplementation, and the establishment of cooling systems for high temperature fermentation systems [5]. Furthermore, several ethanol producing microorganisms used in industry, such as *Saccharomyces cerevisiae*, have strains that are not naturally able to utilize starch and they require high amounts of amylolytic enzymes, which is associated with high cost and impractical ethanol production [12]. Therefore, while ethanol production from starchy materials is racing ahead, the conventional processes used today are not favorable at the economic level; hence, improved methods are desired with microbial strains that enable efficiency and lead to high yield ethanol production in a cost-effective way [4].

A vast amount of microbial species to obtain ethanol from starch in the fermentation process have been presented in the literature. Regardless of the species, parental microorganisms remain insufficient in conventional ethanol production due to the lack of availability of sugar rich input and low ethanol yields. Over 150 amylolytic yeast strains have been reported to be impractical in industrial use because of limited characteristics [13]. Although *Clostridium* spp. and *Zymomonas mobilis* are popular bacteria for ethanol production [14,15], *S. cerevisiae*, well-known and widely used yeast in alcoholic beverages and bakery industries, is traditionally preferred because it sustains steady-state production, can consume various monomeric sugar molecules, has a high fermentation capacity, ethanol productivity and ethanol tolerance, along with having "generally regarded as safe" (GRAS) status [16].

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S. cerevisiae is not naturally able to ferment starch, the development of genetically modified S. cerevisiae strains displaying starch utilizing enzymes (α-amylase and glucoamylase), leading to saccharification and fermentation of starch at the same time has been an area of interest. In this review, the genetic engineering of S. cerevisiae to allow it to utilize starchy sources resulting in a decreased total cost of fermentation and the realization of high ethanol levels will be discussed in detail.

Starch Fermentation

Starch is a cheap, renewable, and fermentable carbon source [17] found in all green plants in various amounts. There is approximately a starch content of 70% in corn, triticale and cassava [18]. Starch molecules are generally associated with lipids, proteins and fatty acids, and the type of association determines the pre-treatment process to be applied before fermentation [19]. Starch consists of glucose monomers joined by glycosidic bonds (α-1,4 glucan-linked D-glucopyranose chains) [18]. It is mainly composed of the linear glucose polymer amylose (20-30%) and highly branched amylopectin (70-80%) (Figure 2) [20]. Amylose has mostly α-1,4-linked D-glucopyranose and sparse α-1,6-linked D-glucopyranose chains (about 0.3% to 0.5%), and forms a flexible molecule leading to interaction with fatty acids, alcohols, and iodine [21]. Amylopectin is a highly branched helix like structured molecule consisting of α-1,4-D-glucopyranose chains [22]. Amylose and amylopectin, found in the starch structure at different rates, form an interconnected structure which directly determines the chemical characteristic of biomass and fermentation yield [23].

Fermentation of starch commonly involves two stages; i) starch hydrolysis by amylolytic enzymes (liquefaction) and saccharification, ii) fermentation of glucose into ethanol [24]. For an efficient starch fermentation, both α-1,4 and α-1,6-debranching hydrolases, with amylases and glucoamylases, and α-glucosidases displaying both α-1,4 and α-1,6-debranching activities are required [25]. As starch itself is not readily utilisable by S. cerevisiae, it has to be hydrolyzed by acid treatment and saccharificated by enzyme (amylose and/or glucoamylase) treatment before the main fermentation process [7]. This is a relatively expensive application as 30-40% of the total cost for the fermentation process is spent for the liquefaction and saccharification necessary for this popular microbe to be used [26]. Moreover, adding caustic soda, lime and sulphuric acid to maintain optimum pH levels for the enzymes, also increases the total cost [27]. Cold starch hydrolysis is an alternative method to decrease the total energy input. However, total enzyme requirement is much higher in cold hydrolysis than starch hydrolysis at high temperatures [28,29]. Although elevated temperatures in the initial step is advantageous to prevent bacterial contamination, high cooling cost result and become a major problem for fermentation because the temperature for industrially available yeasts should be stabilized to 30-37°C. Co-culture systems (amylolytic microorganisms and yeast) have emerged in the SSF process to avoid pure enzyme requirements [30-32]. However, the process is still expensive and the ethanol yield is not satisfactory because of the high starch content consumed for the growth of amylolytic microorganisms. Consolidated Bioprocessing (CBP), hydrolysis and fermentation of sugar molecules in a single step using microorganisms with fermentation capability, is the most effective method for ethanol production from starch fermentation. In this concept, if a microorganism does not naturally express amylolytic enzymes, genetic engineering tools offer a solution for starch fermentation by non-amylolytic microorganisms, e.g. S. cerevisiae as explained in this review [18]. The optimization of temperature requirements for saccharification and fermentation processes and the development of genetically modified S. cerevisiae strains that do not need the addition of exogenous enzymes to achieve the full potential for starch fermentation in a single step, are highly
and soluble starch was attained at high performance levels (0.23 g.l⁻¹.h⁻¹). S. cerevisiae transferred to genome and hydrolyzation of unprocessed starch is required for sufficient starch fermentation by glucoamylase secreting S. cerevisiae, in anaerobic or minimal aerobic conditions [7,34,35,44], a recombinant strain of S. cerevisiae (YF207/pGA11) expressing cell surface anchored R. oryzae glucoamylase has been tested for ethanol production under aerobic conditions (dissolved oxygen was 2.0 ppm), using soluble starch [4]. A high ethanol production rate (0.71 g.h⁻¹) was achieved and the fermentation process has been completed in seven repeated fermentations over 300 h without losing modified gene activity (9×10⁻⁹ U/cell). From a different point of view, as raw and naive starch is not favorable from a commercial perspective, Kosugi et al. have designed an experiment to produce ethanol from cassava pulp rich in starch (up to 60%) [47]. A high production rate of ethanol from 13% (v/v) ethanol yield was obtained from direct hydrolysis of starch via using glucoamylase expressing yeast [36]. The first step of the process began by constructing recombinant strains that express amylolytic enzymes (α-amylase or glucoamylase) for the liquefaction and saccharification of the polymers ends to release β-D-glucose units and saccharification of the starchy biomass. Glucoamylase (1,4-α-D-glucan glucohydrolase; EC 3.2.1.3) is recognized as the most important enzyme which is responsible for the progressive hydrolysis of starch from non-reducing ends to release β-D-glucose units and saccharification of the polymers [37]. The glucoamylase cDNA gene (gla) from Aspergillus awamori has been successfully incorporated into S. cerevisiae genome for the utilization of starch and recombinant strains have been found to be stable for 50 generations without applying any selective pressure [38]. In another study, the glucoamylase enzyme coding sequence was transferred to S. cerevisiae genome and hydrolyzation of unprocessed and soluble starch was attained at high performance levels (0.23 g.l⁻¹.h⁻¹) [39]. The same research group also increased the enzyme activity of the A. awamori glucoamylase by codon-optimizing, compared to native glucoamylase (791 nkat and 591 nkat per gram dry cell weight, respectively) and transformed the recombinant gene to industrial S. cerevisiae (27P) strain for a direct starch fermentation [27]. Although co-culture of S. cerevisiae with an amylolytic microorganism is a conventional option, Nakamura et al. have reported that a recombinant S. cerevisiae, SR93, modified to express glucoamylase has produced more ethanol (24.9 g.l⁻¹) as compared to a co-culture of A. awamori and wild type S. cerevisiae system (22.0 g.l⁻¹), because SR93 has saved the internal starch amount consumed for the growth of the amylolytic microorganism, A. awamori [7]. However, SR93 could not degrade all the starch content efficiently because recombinant glucoamylase enzyme originated from Saccharomyces cerevisiae var. diastaticus was not able to degrade α-1,6 glycoside bond of amylopectin units. It has been recognized that this glucoamylase enzyme, coded by the STA1 gene, lacked a starch-binding domain which made fermentation and ethanol production unsatisfactory [40,41]. Therefore, the starch-binding domain of the Aspergillus niger glucoamylase gene has been fused with STA1 gene resulting in a remarkable hydrolysis and utilization of insoluble starch [42]. Instead of using a starch binding domain, the gene for glucoamylase of Rhizopus oryzae, capable of breaking down both α-1,4- and α-1,6-glycosidic bond efficiently, has been transferred to S. cerevisiae and approximately 80% of starch content was utilized in a 100h fermentation period [43]. As a long fermentation period (~150h) is required for sufficient starch fermentation by glucoamylase secreting S. cerevisiae, it was achieved and the fermentation process has been completed in seven repeated fermentations over 300 h without losing modified gene activity. As the same enzyme from various sources would provide different outcomes due to their divergent kinetic properties and activities in different experimental conditions, determining optimal enzyme source to be transferred is one of the most critical issues. For instance, R. arrhizus glucoamylase gene has been transferred to S. cerevisiae and up to 5% ethanol and 2400 U.l⁻¹ enzyme activity (one of the highest level reported to date) has been obtained in a flask ferment experiment [45]. S. cerevisiae has been modified by Aspergillus oryzae glucoamylases, encoded by glaA and glaB, and R. oryzae glucoamylase separately on the cell surface and compared with each other for their starch fermentation ability [46]. The highest ethanol yield has been obtained from glaA glucoamylase expressing yeast (15 g.l⁻¹ in 24 h); although R. oryzae glucoamylase exhibited the highest glucoamylase activity (9×10⁶ U/cell). From a different point of view, as raw and naive starch is not favorable from a commercial perspective, Kosugi et al. have designed an experiment to produce ethanol from cassava pulp rich in starch (up to 60%) [47]. A high production rate of ethanol from...
5% cassava pulp (91% of theoretical value) has been achieved using S. cerevisiae displaying R. oryzae glucoamylase enzyme.

α-amylase expressing S. cerevisiae

Apart from transforming glucomylase into S. cerevisiae for starch hydrolysis and saccharification, α-amylase modification is another important option in recombinant technology for starch fermentation. The α-amylases (EC 3.2.1.1) breakdown starch molecules from the internal α-1,4-bonds of amylose and amylopectin and release dextrins (10-20 glucose units in length), free glucose and maltose units [37]. Wheat α-amylase has been cloned into S. cerevisiae and found secreted into medium successfully as early as 1987 [48]. Since then, amylase enzymes from different sources such as bacterial [49], yeast [50,51], mold [52], barley [53] and rice [54] have been cloned into S. cerevisiae. From the beginning of these studies, optimization of cell growth and enzyme activity has been determined as the key factors for an efficient ethanol production. The selection of the source for the enzyme is a critical issue as the nature and activity of the enzyme should be compatible with the fermentation conditions. It has been shown that decrease in pH, from 5.5 to 4.5 due to yeast growth, in fermentation resulted in a dramatic reduction in Bacillus subtilis α-amylase activity, whereas there was no effect, even a slight increase, on barley α-amylase activity [55]. S. cerevisiae strains expressing recombinant α-amylase genes (LKA1 and LKA2) obtained from Lipomyces kononenkoei have been proven to convert starch directly into ethanol [56]. However, the rate of ethanol production has been found to be low (17.2 g\text{l}^{-1} in 200 h fermentation period) because of the limited starch hydrolysis by recombinant α-amylase was inadequate. Similarly, Ramachandran et al. have reported LKA1 expressing flocculent and non-flocculent S. cerevisiae strains for ethanol production from raw starch [57]. Genetically modified flocculent strains produced higher ethanol levels compared to non-flocculent counterparts (4.61 kg\text{l}^{-1} and 5.1 g\text{l}^{-1}, respectively) in a 90 h fermentation period. However, the general accepted concept is that S. cerevisiae should express a high amount of α-amylase under aerobic conditions for effective starch fermentation [56,58]. As a different strategy, apart from genetically engineered S. cerevisiae that expresses recombinant α-amylase, ethanol production rate sometimes increased by addition of exogenous glucoamylase enzyme [53,59]. Although this is a preferable option for a high starch fermentation rate, it is not economically favorable from an industrial perspective due to the high price of pure enzymes. In this line, scientists have focused on both α-amylase and glucoamylase expressing S. cerevisiae to increase ethanol yield and decrease total cost.

Co-expression of α-amylase and Glucoamylase

In order to increase the rate of ethanol production from starch fermentation by S. cerevisiae, additional genetic manipulations such as co-expressing of α-amylase and glucoamylase have emerged as a latter strategy. Glucoamylase and α-amylase enzymes synergistically enhance the rate of corn and wheat starch hydrolysis with respect to their individual performances [51,60-62]. Hence, scientists have been working on constructing S. cerevisiae strains that express both enzymes. The glucoamylase gene of A. awamori (GA1), Debaryomyces occidentalis glucoamylase (GAM1), and α-amylase (AMY) encoding plasmids have been transformed into industrial S. cerevisiae strain for a direct conversion of starch to ethanol [63]. Yeast containing GAM1, GA1 and AMY genes have exhibited the highest glucoamylase (required for debranching of starch molecules) activity (1020 U\text{l}^{-1}) compared to only GAM1 or GA1 transformed strains (790 U\text{l}^{-1} and 560 U\text{l}^{-1}, respectively); indicating synergistic activity. Altıntaş et al. have also transformed S. cerevisiae with a bifunctional fusion protein that contained both the B. subtilis α-amylase and the A. awamori glucoamylase, but they have found the biomass (3.86-6.24 g\text{l}^{-1}) and ethanol production (18.4-23.2 g\text{l}^{-1}) insufficient in the experimental model of intermittent starch feeding system [64]. In a different study, three different recombinant strains of S. cerevisiae have been used for the comparison of their ethanol production capabilities [44]. YPG/AB strain expresses B. subtilis α-amylase and the A. awamori glucoamylase separately, whereas YPB-G strain expresses both enzymes as a fusion protein. One last strain YPG/MM expresses mouse α-amylase and A. awamori glucoamylase. YPG/AB strains were found to be superior in ethanol production (43.8 g\text{l}^{-1}) than YPB-G (35.2 g\text{l}^{-1}) and YPG/MM (24.3 g\text{l}^{-1}) strains. Although the glucoamylase activity in YPG/AB and YPB-G strains were similar (1053 U\text{l}^{-1} and 1100 U\text{l}^{-1}, respectively), α-amylase of YPG/AB showed 2.2 fold higher activity than the fusion protein group which was the possible explanation for the higher rate of ethanol production. In addition, low levels of ethanol produced by YPG/MM strain have been attributed to negligible glucoamylase activity. From a different point of view, expressing amylase and glucoamylase genes together with bacterial pullulanase in S. cerevisiae resulted in complete (99%) utilization of the initial starch [65]. However, as it was mentioned in the previous section, using laboratory strains and raw starch resources are not economically favorable and do not always reflect real conditions. In this sense, scientists have tried to optimize starch fermentation using unprocessed biomass and industrial yeast strains. In a high-yielding brown rice fermentation, yeast strain expressing α-amylase and glucoamylase exhibited acceptable ethanol production rate (1.1 g\text{l}^{-1}.h^{-1}) [66]. Industrial yeast strains have also been genetically modified for starch hydrolysis due to their high ethanol production rate and tolerance to harsh conditions. Viktor et al. have tested ethanol production capacities of Aspergillus tubingenis T8.4 α-amylase and glucoamylase expressing laboratory strain, S. cerevisiae Y294, and the semi-industrial strain, S. cerevisiae Mnu1. Y294 and Mnu1 strains have produced 9.03 and 6.67 g\text{l}^{-1} ethanol, respectively, from a substrate load of 200 g\text{l}^{-1} raw corn starch after 10 days fermentation period without any heat treatment [37]. Industrial strains of S. cerevisiae have been transformed with amylase and glucoamylase genes separately and co-cultured for an efficient one-step starch utilization [67]. Activities for glucoamylase and α-amylase have been determined as 920 U\text{l}^{-1} and 7960 U\text{l}^{-1}, respectively.

Cell surface anchored enzyme expressing S. cerevisiae strains

Co-expressing of starch utilizing enzymes has also been studied by anchoring enzymes on the cell wall of yeast to provide long term durability. Expressing on the cell surface and secreting to the medium has various advantages and disadvantages. Secreting of the enzymes to the fermentation environment increases the rate and amount of ethanol by providing high possibility of interaction between starch molecules and enzymes [68]. On the other hand, secreting to the environment is not favorable because of the loss of stability in the early stage of fermentation. An additional consideration for secreted enzymes is including extra ingredients such as metal ions or surfactants in the fermentation medium to provide enzyme stability for repeated large-scale productions. For example, calcium ions have been claimed to be protective for α-amylase during repeated 10-cycles of raw starch fermentation [69]. However, cell surface engineering of yeast for starch utilizing enzymes is suitable for long term stability and repeated large-scale production without the necessity of adding additional reagents. In a recent study, S. cerevisiae strain co-expressing glucoamylase
and α-amylase on the cell surface has been used in 23 continuous cycles of ethanol fermentation without losing enzyme activity [70]. Recombinant yeast strains, glucoamylase and α-amylase anchored on the cell wall (or secreted to the medium), have been developed for a direct starch utilization [71]. Cell surface glucoamylase and α-amylase expressing S. cerevisiae strain has produced more ethanol (60 g l⁻¹ in a 100 h fermentation period) and a higher starch degradation rate than the only cell surface glucoamylase expressing strain (50 g l⁻¹ in 120 h fermentation). In another study, S. cerevisiae co-displaying glucoamylase of R. oryzae and α-amylase of Streptococcus bovis anchored to cell membrane via C-terminal-half region of α-agglutinin and the flocculation functional domain of Flo1p, respectively, has been effective (61.8 g l⁻¹ ethanol in a 72 h fermentation period) in raw corn starch hydrolysis [12]. A recent study has brought a different point of view to starch conversion into ethanol by S. cerevisiae. A recombinant strain of yeast which expressed cell surface engineered aspartic protease has been constructed for the consuming of complex nitrogenous materials, other than starchy molecules found in crops, that resulted in high ethanol yield and shorter fermentation duration (3.8 ± 0.15% improvement in the final ethanol concentration compared to parental strain in 72 h fermentation period) [72].

**Stable Starch Fermenting S. cerevisiae Strains**

Although genetic manipulation is a relatively easy process by using episomal vectors which do not require integrative sites, the stability of the episomal plasmid is a serious problem for long-term and repeated fermentation such that plasmid leakage is generally observed in long-term incubation [7,73]. Gene integration into the microbial genome has been undertaken in many studies to provide long-term enzyme activity [38,74,75]. The δ-sequences of the Ty retrotransposon, or rDNA has been undertaken in many studies to provide long-term enzyme activity [38,74,75]. The δ-sequences of the Ty retrotransposon, or rDNA has been undertaken in many studies to provide long-term enzyme activity [38,74,75]. The δ-sequences of the Ty retrotransposon, or rDNA has been undertaken in many studies to provide long-term enzyme activity [38,74,75]. The δ-sequences of the Ty retrotransposon, or rDNA has been undertaken in many studies to provide long-term enzyme activity [38,74,75].

Enzyme activities could be increased sequence, of activity [38,74,75]. The δ-sequences of the Ty retrotransposon, or rDNA has been undertaken in many studies to provide long-term enzyme activity [38,74,75]. The δ-sequences of the Ty retrotransposon, or rDNA has been undertaken in many studies to provide long-term enzyme activity [38,74,75]. The δ-sequences of the Ty retrotransposon, or rDNA has been undertaken in many studies to provide long-term enzyme activity [38,74,75].

**Table 1: Genetic modifications on S. cerevisiae for high efficient ethanol fermentation.**

| Engineered Enzyme | Gene Source | Cultivation Time (h) | Ethanol Yield (g.l⁻¹.h⁻¹) | Enzyme Activity | Starch Source | Reference |
|--------------------|-------------|----------------------|---------------------------|----------------|--------------|-----------|
| a-aminolase        | Lipomyces kononenkoeae | 90                   | 0.05                      | 87 U.l⁻¹       | Raw corn starch | [57]      |
| Glucoamylase       | Aspergillus awamori  | NA                   | ND*                       | 162 U.l⁻¹      | Soluble starch  | [38]      |
| Glucoamylase       | Aspergillus awamori  | 50                   | 0.23                      | 624 U.l⁻¹      | Soluble starch  | [39]      |
| Glucoamylase       | Saccharomyces diastaticus (var. diastaticus) | 200           | 0.66                      | ND             | Soluble starch  | [7]       |
| Glucoamylase       | Rhizopus oryzae     | 168                  | 0.77                      | 60.2 U.g⁻¹      | Cassava pulp rich in starch (60%) | [47] |
| Glucoamylase       | Aspergillus awamori  | 80                   | 0.175                     | ND             | Soluble starch  | [58]      |
| Glucoamylase/ a-amylase | Rhizopus oryzae/Saccharomyces cerevisiae | 72                   | 0.85                      | 57/114 U.g⁻¹    | Raw corn starch  | [12]      |
| Glucoamylase/ a-amylase | Saccharomyces cerevisiae/ Lipomyces kononenkoeae | 120             | 0.178                     | 1340 /<30 U.l⁻¹ | Soluble starch  | [56]      |
| Glucoamylase/ a-amylase | Rhizopus oryzae/ Streptococcus bovis | 120              | 0.74                      | 790/1306 U.l⁻¹ | Raw corn starch  | [70]      |
| Glucoamylase/ a-amylase | Rhizopus oryzae/Saccharomyces cerevisiae | 24               | 1.2                       | 4700/1800 U.l⁻¹ | High-yielding rice | [66]      |
| a-amylase/ glucoamylase/ glucoamylase with debunching activity | Debaryomyces occidentalis/ Aspergillus awamori/ Debaryomyces occidentalis | 168               | 0.45                      | 5940/1020 U.l⁻¹ | Soluble starch  | [63]      |
| Glucoamylase/ isoamylase | Aspergillus awamori/ Pseudomonas amylophila | 140              | 0.137                     | ND             | Soluble starch  | [81]      |

*NA: Not available,  
*ND: Not determined

The δ-integration sequence with respect to the integration of a recombinant gene. Enzyme activities could be increased sequence, of activity [38,74,75]. The δ-sequences of the Ty retrotransposon, or rDNA has been undertaken in many studies to provide long-term enzyme activity [38,74,75]. The δ-sequences of the Ty retrotransposon, or rDNA has been undertaken in many studies to provide long-term enzyme activity [38,74,75].

Energy prices are growing fast, mainly because of depletion of petroleum sources. Therefore, alternative energy sources obtained from sun, wind and biomass have become attractive recently. Ethanol, produced by fermentation from biomass, as a promising alternative energy is a transportable and economically favorable source. In addition, ethanol production from various feedstocks such as starch maintains a considerable potential due to their availability, accessibility and relatively low cost in comparison to surose and glucose based feedstocks. Recombinant DNA technology offers a valuable opportunity for consolidated bioprocessing processes of the biomass fermentation. To date however, transforming new pathways and overexpression of a single or group of enzymes have...
not taken biomass fermentation via \textit{S. cerevisiae} to a satisfactory level for industrial arena. Although there are hundreds of studies claiming higher ethanol production from starchy materials, most of these have been tested at laboratory scale only and not at large-scale (Table 1). Several laboratory yeast strains have been proven to exhibit remarkable starch fermenting capacity and produce high amounts of ethanol, but transferring engineering technology to industrial \textit{S. cerevisiae} strains has remained insufficient. They are not convenient for repeated large scale applications because of low stability of the modification, high cost of the process, low yield of ethanol production and fermentation rate. Although using exogenous amylase and glucoamylase seems to increase total yield, developing yeast strains sufficient alone for the whole fermentation process is desirable. New amylase and glucoamylase from different sources should be presented and investigated for their activity (alone or in combination) and compatibility with industrial yeast stains. Researchers should continue to conduct studies on two main areas; efficient hydrolysis and fermentation of biomass, which result in high ethanol production rate and combination of improved bioprocess applications with genetic engineering tools in the view of productivity and large scale processes.

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