AIMS Energy, 6(6): 979–992.
DOI: 10.3934/energy.2018.6.979
Received: 05 September 2018
Accepted: 11 November 2018
Published: 14 November 2018

http://www.aimspress.com/journal/energy

Review

Bioethanol synthesis for fuel or beverages from the processing of agri-food by-products and natural biomass using economical and purposely modified biocatalytic systems

Divakar Dahiya and Poonam Singh Nigam*

Biomedical Sciences Research Institute, Ulster University, Coleraine, Northern Ireland, UK

* **Correspondence:** Email: p. singh@ulster.ac.uk.

Abstract: This review describes the role of suitable and modified microorganisms as economical biocatalysts in the processing of by-products generated in industries and agriculture, which are cheaply available globally as renewable resources. Since the microbial processing can be economically used to produce a variety of value-added products, by employing specific species of microorganisms as biocatalysts; but to be specific to the title of this review the information included in this article has only emphasized on one important consumer-product bioethanol. The conclusion of the information gathered in this review is that, the selection and modification of a microbial biocatalyst should be strategically done. For example: employing an yeast strain of Saccharomyces or a non-Saccharomyces culture, is important in bioethanol synthesis; the optimisation of biocatalyst is also important according to the type of material being processed in the system as it could be a by-product or waste residue of agriculture, food & beverage industry or simply the seasonal locally available fruits. The other information, which has been included in this review, is on the modification of biocatalysts and important factors influencing the efficiency of bioprocessing, for the necessity of economical yield of bioethanol.

Keywords: bioethanol; agro food wastes; biocatalyst; bioprocessing; immobilisation; yeasts

1. Introduction

Bioethanol has many applications, as a source of biofuel for energy and in speciality alcoholic

beverages, depending on the source of raw material used in its synthesis. A variety of microorganisms has been employed as suitable biocatalysts for the synthesis of bioethanol. The use of a specific microorganism depends on the type of substrate cheaply and locally available for the bioprocessing or bioconversion. In different countries, different types of renewable substrates are annually produced; the list of such substrates is very long but it mainly includes by-products and residues generated from agriculture, food and beverage industries [1]. The cheaply available by-products from sugar industry are sugarcane bagasse, sugar beet pulp and molasses recovered after the sugar production; from dairy industry large volume of whey and lactose containing waste-water effluent is generated in the process of cheese production; from agriculture and farming industry discarded grains of non-consumption quality, damaged cereals, cellulosic materials, starchy wastes, straw, and cobs etc are generated annually [2,3]. These substrates can be bio-converted into fuel ethanol, an energy-yielding product. The expensive grade bioethanol is mainly used as a beverage consumer-product, which is produced worldwide as alcoholic drinks and mixed beverages for direct human consumption, using regionally produced seasonal sweet fruits [4–6].

For an efficient bioconversion of a specific substrate in the production process for bioethanol, a biocatalyst used is a specifically selected strain of microorganism. The bioethanol produced from different substrates may have potable and non-potable applications. For instance, the bioethanol produced from a variety of sweet fruits, have large market for sale as alcoholic beverages and specialised fruit-wines [4–6], and whereas bioethanol produced from the microbial-conversion of ligno-cellulosic renewable biomass has industrial application as an economical source of fuel and non-conventional energy [7,8].

2. Bioethanol from the processing of substrates by strategic selection of microbial-biocatalysts

The microorganisms, whether endogenous and exogenous, are selected for the biosynthesis of ethanol, according to the composition of substrates available for bioprocessing. The exogenous microorganism is a specific pure species of a microbial-culture, whereas the endogenous microbiota are a variety of strains contributed by the natural substrate itself, such as from the damaged skin and bruised surfaces, particularly when using grapes, berries and other seasonal soft fruits. The quality of bioethanol produced is controlled by the microorganisms involved in the fermentation process, and by the contributions made by the type of microorganisms, such as yeasts alone or yeasts with bacteria.

2.1. Bioethanol by microbial conversion of molasses

Molasses is produced in sugar industry as a large amount of by-product after the crystallisation of sugar, which contains high level of fermentable sugar. Suitable strains of yeast have been employed to utilise this sugar content to convert it into bioethanol [7,8]. In one such process optimised for increased yield of bioethanol, the sugarcane molasses was pre-treated with Amberlite and non-living biomass [9]. Research project involved using *Saccharomyces cerevisiae* yeast, where cells were immobilised on alginate matrix for their long-term use in a continuous fermentation process using molasses as a raw material for a steady yield of bioethanol [10]. Researchers have also tried to immobilise actively growing yeast cells in a column reactor and use them in the

continuous-fermentation process of bioethanol production from sugarcane molasses [11].

The climate is usually warmer in those countries, where sugarcane molasses is produced in sugar mills and mostly used in distilleries for bioethanol production. The temperature normally increases in summer period higher than 37 ºC, which is the optimum alcohol fermentation temperature for yeasts. Therefore, the fermentation efficiency of the normal ethanol-producing yeasts drops down during hot seasons. As a solution to this problem, research was conducted using a thermotolerant isolate of yeast strain *Kluyveromyces marxianus* to ferment molasses at temperature higher than 37 ºC, which could be used in warm climatic conditions. The experiments were also conducted using thermotolerant yeast by immobilising its cells within alginate matrix and using this catalytic system for ethanol fermentation [12]. An industrial scale trial was also conducted using thermotolerant strains of yeast in summer months at high temperature climatic conditions [13]. A selected strain of thermotolerant yeast *K. marxianus* was used for pilot-scale trial in an Indian distillery in hot months of summer, to avoid water-cooling of production-scale large fermenters [14]. Since the good yield of bioethanol was not possible in summer months with the use of conventional distillers' yeast *Saccharomyces cerevisiae,* which ferments molasses optimally at 37 ºC, unless the temperature of fermenters was controlled using expensive cooling system. Thus with the use of thermotorant yeasts at temperature 45 ºC, the overall cost of alcohol production could be lowered by eliminating cooling-cost of large scale fermenters in distilleries [14].

2.2. Bioethanol by microbial conversion of whey and lactose containing effluents

Commercial production of cheese generates a liquid by-product in large volumes. This wastewater is whey and it is rich in lactose sugar. Experiments were conducted in detail to utilise the sugar content present in whey for bioethanol production, and it was reported that the thermotolerant yeast strain was capable of fermenting lactose into bioethanol at high temperature using lactose containing medium [15,16]. De-lignified cellulosic were tried as a low-cost support material to immobilise yeast cells to ferment whey [15,17].

The process has been optimised using a tested strain of thermotolerant yeast, *K. marxianus to* utilise this valuable resource generated generated in large volumes as an effluent in dairy industry to produce ethanol [18–20]. The fermentation efficiency of yeast could be improved by using the cells immobilised in alginate matrix [18], and in magnetically responsive matrices [19]. One research group has reported that supplementation of Mn+ in lactose-fermentation medium stimulated the activity of yeast cells, and thus the overall bioethanol synthesis could be optimised for better yields from a by-product whey [20].

2.3. Bioethanol by microbial conversion of ligno-cellulosic substrates

Agriculture produces large quantities of residual substrates like sugarcane bagasse, wheat-straw, corn cob, sorghum and tapioca etc. Distilleries and breweries worldwide use barley as the starting material for ethanol production and after malting process a solid by-product is generated containing spent barley grains. All these by-products contain fermentable carbohydrates like cellulose about 40–50% and hemicellulose 20–25%. The overall 70–75% of carbohydrates available in by-products and residues could be utilised as a cheaper source of fermentable sugars. In such an experimentation, bioethanol was produced using sugars obtained after the enzymatic saccharification

of these cheaper agricultural residual-materials [21,22]. Bioconversion studies were conducted where researchers have tried different strains of microorganisms, as suitable and economical biocatalysts to utilize such annually renewable substrates for economical bioethanol production. The alcohol-yields were optimised for improved fermentation-efficiencies by designing suitable processing systems, such as batch fermentation and fed-batch processes [23].

2.4. Bioethanol by microbial conversion of apple-must

In apple growing areas where this natural substrate is available annually at a lower cost, it is used for making a consumer product beverage, cider. The fermentation of apple-must might use the wild micro-flora present on the surface of fruit, and other yeasts in wineries, which are indigenous. The product of such type of fermentation is the traditional cider produced by the indigenous organisms, which are naturally present on surface of fruit-apple. Different countries have their own cider making process, such as the apple cider produced in France employs the complex natural micro-flora, which are necessary for partial and slow incomplete fermentation of sugars. Whereas, in Brazil cider from apples is produced using specific yeasts such as, *Saccharomyces cerevisiae* var. *uvarum*, *Metschnokowia pulcherrima, Hanseniaspora valbyensis,* and *Brettanomyces* sp. Usually the process of traditional fermentation of apple-must does not perform the inoculation step of exogenous specific yeast strains, and the apple juice is initially fermented by dominating non-Saccharomyces species, which is essential to produce specific flavours in cider [24,25]. But the initial natural micro-flora does not exist longer, as the bioethanol concentration rises in the production system. It has been found that at a later stage of process the yeast *Saccharomyces cerevisiae* var. uvarum dominates and as a result wine-flavour is contributed in the product. The process of traditional cider generally continues in a secondary fermentation or maturation, which is known as malo-lactic fermentation, completed by bacterial strains *Lactobacillus* spp. and *Leuconostoc* spp [4–6].There are reports [26] application of *oenococcus* in its immobilised form rather using free cells for controlling malolactic fermentation.

There is a common practice for commercial standardised production of this beverage to employ naturally selected *Saccharomyces* strains [27]. Therefore, two strains of *Saccharomyces, bayanus* and *cerevisiae* were used for making of cider in Spain. The final product ethanol containing cider produced by use of *S. bayanus* contained two polypeptides of high molecular weight and two peptides of medium molecular weight. Whereas ciders produced by the use of *S. cerevisiae* contained two polypeptides of medium molecular weight and two of low molecular weight. These polypeptides played an important role in controlling the high and lower foam-stability of ciders, respectively.

The continuous fermentation of apple juice to improve technological and sensory qualities of cider has been tried by using co-immobilised yeast *S. bayanus* and bacteria cells of *Leuconostoc oenos* immobilised in alginate matrix [28]. The continuous process reduces the fermentation time of apple juice necessary for a better control on flavour formation, which is not possible in a batch fermentation system retaining apple juice for longer periods.

2.5. Bioethanol by microbial conversion of seasonal fruits

Specific bioethanol-products are made using selected sweet fruits, which are available cheaper in the area of their cultivation during seasons. The pulp of fruits contains sugars and organic acids,

which produce volatile and phenolic compounds in product [4–6]. In bioethanol synthesis the composition of fruits contributes to the desired sensory quality of final product, and accordingly the type of fruit is selected for a specific product such as apples for making cider; and peaches and berries are used for making specialised alcoholic beverages [6]. For this purpose commercial preparation of wine-yeasts are used in modern wineries to prepare specialised wines for commercial production [5,6].

In experiments of alcohol production done by several researchers, certain fruits are reported to produce better quality alcoholic-beverages due to maximised colour and flavour in fruit pulps e.g. plums [29,30], papaya [31], and mango [32]. In all fruit fermentations, *S. cerevisiae* is the principal organism to carry most of the fermentations. In some studies, yeasts cells in free-state and in immobilised form, have been employed for alcoholic-beverage production [33]. Usually indigenous strains of *S. cerevisiae* are significant in contributing sensory properties but the use of an exogenous specific strain determines the quality of fermentation by reducing the occurrence of problems associated with unwanted organisms. The activities of micro-flora involved in ethanol fermentation control the organo-leptic properties of the product. The metabolic activities of both endogenous and exogenous microorganisms contribute to oenological properties, for their involvement in whole process of main fermentation and also post-fermentation [4–6].

3. Important catalysis factor in the process of bioethanol synthesis

Though the total sugar level in fermentation system is responsible for the yield of bioethanol produced, but there is one very important environmental-factor of the system, the temperature of fermentation process. Since temperature regulates the overall growth and metabolism of microorganism in system and hence, the final yield of bioethanol is also affected. Temperature has a great impact on fermentation efficiency of microorganisms and hence on its kinetics [7,34,35]. Temperature can have an important role in the existence of indigenous yeast and on yeasts ecology. There is a report on using a cryo-tolerant strain of *S. cerevisiae* at extremely low temperatures to successfully perform the fermentation of maltose containing medium [36]. Temperature plays most important role in controlling yeasts' respiratory and fermentation efficiency. The optimum temperature of ethanol synthesis is different for different strains of yeasts. The bioethanol producing yeast have capability of transforming 10% more sugar for each temperature increase of 1 °C in the same elapsed fermentation time.

The temperature maintained in the production system is also responsible for the progression of fermentation at constant speed. At lower or higher than optimum temperature for the growth and metabolic activities of yeast, the fermentation goes slow and eventually stops before all sugars are fermented. Therefore, to overcome the problem of increased temperature in hot climatic conditions and sensitivity of normal yeast strains, affecting the fermentation efficiency, the thermotolerant yeasts have been isolated. These strains were screened in lab for their capabilities growing at 52 °C [34] and producing optimum concentration of ethanol at 45 °C and 50 °C, and then these thermotolerant isolates were used at large scale [13]. Lactic acid bacteria (LAB) are now considered as the starter cultures for malo-lactic fermentation [37]. Genisheva et al. [38] employed LAB in their immobilized form for malo-lactic fermentation.

4. Bioethanol synthesis employing immobilized microorganisms as biocatalyst

Microorganisms, to perform as catalysts in their immobilised form [39], have been studied for two aspects: Firstly their performance to achieve a better yield of ethanol, and secondly production of bioethanol in a continuous process [18–20,40]. For the immobilisation of microorganism, two types of supports were used to immobilise [41,42] high temperature-tolerant yeast. Delignified cellulosic material (DCM) has been successfully used to immobilise *Lactobacillus casei* cells and has been employed for malolactic fermentation (MLF) [43]. The research reports that the ethanol was produced in first stage of process employing yeast cells immobilized on DCM at lower temperature 20 °C, and after the completion of bioethanol production, in second stage malolactic fermentation was started using immobilized *L. casei* cells at 27 °C [43].

The immobilised microbial cells have been purposely designed for the recycling of these re-usable biocatalysts in continuous fermentation systems to make overall process economical. In one such continuous process running for one month, up to eleven repeated batches of bioethanol production and subsequent MLF were successfully performed. In these experiments after few repeats of batches, the MLF activity of the immobilized biocatalyst was down. The decrease in activity was noticed from 80 to 2% in malic acid degradation, by 0.5–0.1 unit in pH, acetic acid remained stable (0.002 g/L), and the higher ethanols, 1-propanol, isobutyl ethanol, and amyl ethanol were decreased by 84, 23, and 11%, respectively, and ethyl acetate concentration was increased by 56% [43].

The rate of bioethanol synthesis could be significantly increased using yeast immobilised on tubular cellulose (TC) as carrier [17]. In this type of immobilisation, yeast cells adhered on the surfaces of tubular cellulose by physical adsorption due to electrostatic and other weak forces, and many cells were entrapped into the cavities of the carrier surface. TC was selected as an effective support for the immobilisation of yeast cells, as it has a number of advantages such as its global-availability, low cost, and suitability for extremely low temperature bioethanol fermentation. This technology has been developed for low-temperature bioethanol production using immobilised yeasts, and the fermentation efficiencies have been compared with the process performed using free cells of yeast [36]. Ca-alginate gel has been used to immobilise cells of *Schizosaccharomyces pombe* cells by entrapment method, these cells were used in continuous fermentation process conducted at different dilution rates to evaluate the extent of malic acid degradation. Gel-entrapped cells were found effective in the de-acidification of wine without affecting the analytical profiles of the product [44].

5. Bioethanol synthesis employing mixed microorganisms in same process

A successful process of simultaneous saccharification and fermentation, combining the hydrolysis of starch and simultaneous fermentation of released sugars to bioethanol was carried out in same system [45]. Simultaneous bioethanol and malolactic wine fermentations were studied to conduct in same process, where *Saccharomyces cerevisiae* and *Oenococcus oeni* have been immobilized together in the layers of a cellulose/starch gel composite [46]. The purpose of co-immobilization of two different microorganisms is to conduct two bioprocesses in one bioreactor, simultaneously lowering the overall cost production and investment. This is anticipated that there could be a competition between yeasts and lactic acid bacteria (LAB), which may cause problem of a slow process due to inhibition. A solution to this issue was thought as separate entrapment of two

types of microbial cells in different matrices to formulate a composite biocatalyst. This hypothesis was used in an European-collaborative project, where research team investigated the simultaneous bioethanol and malolactic fermentations of grape must [46].

Several organic natural materials, such as cellulosic residues, cereal grains, pieces of different fruits, and starch have been studied as support materials for the immobilization of yeasts and bacteria for bioethanol fermentation. The delignified cellulosic materials (DCM) have been used as immobilization support in wine making, with their favorable effect on bioethanol production and MLF, at very low temperatures of wine making [17]. The structure of DCM was studied using scanning electron microscopy, which clearly showed nano-and micro-tubes in DCM. Hence DCM proved to be a good carrier of mixed biocatalysts working effectively in low temperature fermentation and other bioprocesses [17].

The use of bacterial cells of *Oenococcus oeni* immobilized on DCM was useful in developing MLF in wine making. Therefore, a biocatalyst has been purposely designed by co-immobilising yeast *Saccharomyces cerevisiae* and bacteria *O. oeni* on separate layers of a composite carrier to achieve additional advantages in wine making [17]. The benefits achieved in overall process were in many aspects including, the increased viabilities of microbial cells, their resistance to low pH and increasing concentration of ethanol, higher cell densities, simultaneous bioethanol and ML fermentation in one bioreactor, and the improvement in the quality of final product-wine. Another two-layer composite consisting of wheat-starch gel and nano-tubular DCM, supported the viability and activity of yeast cells *S. cerevisiae* together with bacterial cells *O. oeni*. Such immobilized bio-catalysts if used for wine making, would be increasing the fermentation efficiency and quality of product [17].

Ethanol production using natural starch as substrate has been studied in a single-step fermentation process by employing a mixed culture of *Saccharomyces diastaticus* and *Saccharomyces cerevisiae* 21 [47]. The maximum ethanol fermentation efficiency was achieved using 6% starch at 93% conversion rate based on theoretical yield. Similarly, in a mixed culture fermentation by *Endomycopsis capsularis* and *S. cerevisiae* 21, the ethanol yield obtained was higher than the yield from a single-culture process employing *E. capsularis.* This was verified when conducting the ethanol production from starch in a two-step process, first starch was hydrolysed by treatment with enzymes α-amylase and glucoamylase, and then the fermentation of saccharified starch by a single culture, the overall fermentation efficiency was found lower [47].

6. Designing biocatalysts for required benefits

Ethanol producing yeasts have been studied for an improvement in qualities desired in their performance. Researchers have specifically engineered yeasts genetically to prepare biocatalysts having the property of thermotolerance [48]. The technique sought in such genetic manipulation is based on—to isolate specific genes from a genome and to introduce it into yeast cells, resulting in their genetic transformation. Extensive research has been published over years in studies of *S. cerevisiae* in defined media using optimised conditions, where genes associated with desired properties, have been cloned and sequenced, which are relevant to winemaking. The effective methods have been established for the transformation of *S. cerevisiae* and many wine strains [49].

The advanced research has defined new technologies for ethanol production using molecular-biology, application of such techniques have evolved the performance of yeasts e.g. thermotolerant

yeasts efficient in bioethanol synthesis at higher temperature. A promising development in the production of sparkling wines has been achieved with the application of immobilized yeasts cells supported on natural gels of alginate and carrageenan. The recombinant DNA technology is the tool for the improvement of yeasts for their performance affecting the quantity, as well as the quality of final product. One such designing of efficient biocatalysts was achieved where strains of yeasts with thermotolerance property have been developed for their potential use for industrial ethanol production in warm climate conditions [50]. In a very recent work, metabolic engineering strategies have been reported to improve the process of ethanol production in cellulolytic *Saccharomyces cerevisiae* by Song et al. [51], who have claimed that their work will significantly increase our knowledge of how to engineer optimal yeast strains for biofuel production from cellulosic biomass.

Lignocellulosic substrates contain a significant amount about 25% of hemicellulose, a pentose polymer. Therefore, the utilization of xylose in the bioconversion of saccharified-lignocellulosic biomass into ethanol is an important aspect to achieve high yield of alcohol. The non-conventional yeast *Ogataea polymorpha* is thermotolerant and an ideal biocatalyst to bioconvert xylose from hydrolysed hemicellose to ethanol at high fermentation temperatures. Dmytruk et al. [52] have reported that studying the molecular mechanisms of regulation of xylose metabolism is a promising way toward increased xylose conversion to ethanol, and they have found that the autophagy-related gene ATG13 is involved in control of xylose-alcoholic fermentation by the thermotolerant methylotrophic yeast *Ogataea polymorpha* [52].

7. Current industrial-scale producer of ethanol

Enerkem is the first commercial-scale factory in the world for the production of cellulosic ethanol from non-recyclable, non-compostable mixed municipal solid waste. Canadian firm Enerkem Inc has started producing cellulosic ethanol from solid waste at a commercial-scale plant in Edmonton, Alberta. The waste-to-biofuels and chemicals company plans to "progressively increase" production at the facility while it prepares to build more plants domestically and internationally [53]. India has established its first 2G ETHANOL Production Plant in Uttarakhand, India to scale up ethanol production using endogenous feedstock from agricultural wastes [54]. This commercial plant has capacity to bioconvert 10 tons of lignocellulosic material per day into ethanol, the material used in this production plant is a mixture of several agricultural residues generated in India such as, sugarcane bagasse, wheat straw, rice straw, bamboo-stalks, corn stover etc. In Central Europe Clariant opened Germany's largest demonstration-scale cellulosic ethanol production plant in the Bavarian town of Straubing in July 2012. The plant processes agricultural residue as feedstock to produce second-generation bioethanol [55].

8. Conclusion

This review is based on published information available in literature on this subject of bioethanol synthesis for fuel or beverages from the processing of agri-food by-products and natural biomass [56] using economical and purposely modified biocatalysts. This review provides an overview of information on various aspects related to biosynthesis of ethanol, such as microorganisms, and the process development (Tables 1, 2). The conclusion of all studies is that certain process factors have to be optimised in each scenario for improved yield in an economical

process. Considering the fact that the ethanol should be produced using a specific starting material which is cheaply available in the area, without adding any transport charges of importing substrates for ethanol production, biocatalysts are needed to be specifically selected and modified.

Table 1. Bioethanol production for fuel and beverages from the processing of agri-food by-products and natural biomass.

Substrate, Biomass	Biocatalyst (Microorganism)	References
Seasonal Fruits (Locally available in area of processing), Grapes	Saccharomyces yeast strain, non-Saccharomyces yeasts, Bacteria	[4–6]
Sugarcane Molasses, a sugar-rich by-product from sugar Industries		
1. High temperature-fermentation	1. Thermo-tolerant yeast-*Kluyveromyces marxianus*	[7–9,12]
2. Continuous production in Immobilized system	2. Distillers' yeast—*Saccharomyces cerevisiae*	[10]
3. Continuous production in Column Reactor	3. Alginate-immobilized cells of *Saccharomyces cerevisiae* HAU-1	[11]
4. Industrial-scale production in Distillery	4. Thermo-tolerant yeast—*Kluyveromyces marxianus* IMB3	[13,14]
Whey Cheese-industry effluent	1. Thermo-tolerant yeast *Kluyveromyces marxianus*	[15–17]
1. High-temperature alcoholic fermentation of whey	immobilized on de-lignified cellulosic material.	
2. Lactose supplemented medium	2. Alginate-immobilized *Kluyveromyces marxianus*	
3. Manganese (Mn^2) Supplemented lactose medium	3. Magnetically-responsive matrices Immobilized *Kluyveromyces marxianus*	[18]
		[19,20]
Agricultural Biomass		
Sorghum (Millet, Durra, Jowari, Milo)	Distillers' yeast and *Saccharomyces cerevisiae*	[21–23]
Tapioca from *cassava* plant (*Manihot esculenta*)	HAU-1 in Batch and Fed-batch fermentation	
Apple-must (*Malus pumila,* Malus domestica*, Malus sylvestris, Malus communis, Pyrus malus*)	Saccharomyces, non-Saccharomyces, Oenococcus yeasts + Lactic acid Bacteria, co-immobilized yeast and *Leuconostoc oenos*	[4–6,24–28]
Berries, Grape (*Vitis vinifera*)	Standard yeasts, Apiculated yeasts	[6]
Plum musts (*Prunus domestica*)	*S. cerevisiae* and *Schizosaccharomyces pombe* (free and immobilized cultures)	[29,30]
Papaya musts (*Carica papaya*)	*Williopsis saturnus* and *Saccharomyces cerevisiae*	[31]
Mango pulp (*Mangifera indica*)	yeast-mango-peel immobilized biocatalyst system	[32]
Cagaita (*Eugenia dysenterica* DC)	Free and immobilised Standard yeasts	[33]

Table 2. Important catalysis factors and process modifications in the process of bioethanol synthesis.

Catalytic Factors, Modifications	Biocatalyst (Microorganism)	References
High-temperature biosynthesis process	Thermotolerant *Kluyveromyces marxianus* IMB 1-5	[7,34,35]
Low-temperature biosynthesis process	Cryotolerant strain of *Saccharomyces cerevisiae* immobilised on porous cellulosic material	[36]
Production in Batch-Fermentation	Free-floating; and alginate-immobilized thermotolerant yeast strain *Kluyveromyces marxianus*	[39]
Production in Continuous-fermentation	thermotolerant *Kluyveromyces marxianus* immobilized on alginate, magnetically responsive matrices; volcanic-mineral Kissiris	[18–20,40–42]
Continuous-fermentation production	*Lactobacillus casei* cells immobilized on de-lignified cellulosic material	[43]
Continuous de-acidification in alcohol fermentation	immobilized cells of *Schizosaccharomyces pombe*	[44]
Simultaneous raw starch hydrolysis and ethanol fermentation	Glucoamylase from *Rhizoctonia solani* and *Saccharomyces cerevisiae*	[45]
Simultaneous mixed Alcoholic + Malolactic fermentation	Mixed microorganisms *Saccharomyces cerevisiae* + *Oenococcus oeni* immobilised in layered cellulose/starchgel-composite	[46]
Single-step process—Ethanol Production from Raw starch (un-hydrolysed by amylolytic enzymes	Co-culture of amylolytic yeasts and standard yeast *Saccharomyces diastaticus* + *S. cerevisiae* 21; *Endomycopsis capsularis* + *S.cerevisiae* 21	[47]
Genetic manipulation for Improved and Varied qualities for final products	Thermotolerance in mutants of *Saccharomyces cerevisiae*; methylotrophic yeast *Ogataea polymorpha*	[48–52]

Conflict of interest

The authors declare there are no conflicts of interest in this paper.

References

1. Procentese A, Raganati F, Olivieri G, et al. (2017) Renewable feedstocks for biobutanol production by fermentation. *New Biotechnol* 39: 135–140.
2. Procentese A, Johnsona E, Orr V, et al. (2015) Deep eutectic solvent pretreatment and subsequent saccharification of corncob. *Bioresource Technol* 192: 31–36.
3. Procentese A, Raganati F, Olivieri G, et al. (2018) Deep Eutectic Solvents pretreatment of agro-industrial food waste. *Biotechnol Biofuels* 11: 37.

4. Nigam P (2000) Wines: Specific aspects of oenology, In: Robinson RK, Batt CA, Patel PD, Eds., *Encyclopaedia of Food Microbiology*, London: Academic Press, 2316–2323.
5. Nigam P (2011) Microbiology of winemaking, In: Joshi VK, Ed., *Handbook of Enology: Principles and Practices*, Delhi: Asiatech, 383–404.
6. Nigam P (2011) Wines: Production of Special Wines, In: Batt CA, Tortorello ML, Eds., *Encyclopedia of Food Microbiology*, Elsevier Ltd, UK: Academic Press, 793–799.
7. Banat IM, Nigam P, Singh D, et al. (1998) Ethanol production at elevated temperatures and bioethanol concentrations: Part I—yeasts in general. *World J Microb Biot* 14: 809–821.
8. Banat IM, Nigam P, Singh D, et al. (1998) Ethanol production using thermo-tolerant/thermophilic yeast strains: Potential future exploitation, In: Pandey A, Ed., *Advances in Biotechnology*, Educational Publishers & Distributors N, Delhi, India, 105–119.
9. Farrell EA, Bustard M, Gough G, et al. (1998) Ethanol Production at 45 ^{o}C by *Kluyveromyces marxianus* IMB3 during growth on molasses pre-treated with Amberlite® and non-living biomass. *Bioproc Eng* 19: 217–219.
10. Yadav BS, Rani U, Dhamija SS, et al. (1996) Process optimization for continuous ethanol fermentation by alginate-immobilized cells of *Saccharomyces cerevisiae* HAU-1. *J Basic Microb* 36: 205–210.
11. Sheoran A, Yadav BS, Nigam P, et al. (1998) Continuous ethanol production from sugarcane molasses using a column reactor of immobilized *Saccharomyces cerevisiae* HAU-1. *J Basic Microb* 38: 123–128.
12. Gough S, Brady D, Nigam P, et al. (1997) Production of ethanol from molasses at 45 ^{o}C using alginate-immobilized *Kluyveromyces marxianus* IMB3. *Bioproc Eng* 16: 389–392.
13. Abdel-Fattah WR, Fadil M, Nigam P, et al. (2000) Isolation of thermotolerant ethanologenic yeasts and use of selected strains in industrial scale fermentation in an Egyptian distillery. *Biotechnol Bioeng* 68: 531–535.
14. Singh D, Banat IM, Nigam P, et al. (1998) Industrial scale ethanol production using the thermotolerant yeast *Kluyveromyces marxianus* IMB3 in an Indian distillery. *Biotechnol Lett* 20: 753–755.
15. Kourkoutas Y, Dimitropoulou S, Kanellaki M, et al. (2002) High-temperature bioethanolic fermentation of whey using *Kluyveromyces marxianus* IMB3 yeast immobilized on delignified cellulosic material. *Bioresource Technol* 82: 177–181.
16. Kourkoutas Y, Dimitropoulou S, Marchant R, et al. (2001) Whey liquid waste of dairy industry as raw material for fermentation with thermophilic *K. marxianus*. In: Proc of 7th Intl Conf Env Sci Technol, Univ Algean: Lekkas TD Ed Global Nest, 226–233.
17. Koutinas AA, Sypsas V, Kandylis P, et al. (2012) Nano-Tubular Cellulose for Bioprocess Technology Development. *PLoS One* 7: e34350.
18. Brady D, Nigam P, Marchant R, et al. (1997) Ethanol production at 45 ^{o}C by alginate-immobilized *Kluyveromyces marxianus* IMB3 during growth on lactose-containing media. *Bioproc Eng* 16: 101–104.
19. Brady D, Nigam P, Marchant R, et al. (1996) Ethanol production at 45 ^{o}C *by Kluyveromyces marxianus* IMB3 immobilized in magnetically responsive alginate matrices. *Biotechnol Lett* 18: 1213–1216.

20. Brady D, Nigam P, Marchant R, et al. (1997) The effect of Mn^{2+} on ethanol production from lactose using *Kluyveromyces marxianus* IMB3 immobilized in magnetically responsive matrices. *Bioproc Eng* 17: 31–34.
21. Aggarwal NK, Nigam P, Singh D, et al. (2001) Process optimization for the production of sugar for the bioethanol industry from Tapioca, a non-conventional source of starch. *World J Microb Biot* 17:783–787.
22. Aggarwal NK, Nigam P, Singh D, et al. (2001) Process optimization for the production of sugar for the bioethanol industry from sorghum, a non-conventional source of starch. *World J Microb Biot* 17: 411–415.
23. Nigam P, Pandey A (2009) Biotechnology for Agro-Industrial-Residues-Utilisation Springer. ISBN 978-1-4020-9941-0; e-ISBN: 978-1-4020-9942-7.
24. Sánchez A, Coton M, Coton E, et al. (2012) Prevalent lactic acid bacteria in cider cellars and efficiency of *Oenococcus oeni* strains. *Food Microbiol* 32: 32–37.
25. Sánchez A, Rodríguez R, Coton M, et al. (2010) Population dynamics of lactic acid bacteria during spontaneous malolactic fermentation in industrial cider. *Food Res Int* 43: 2101–2107.
26. Herrero M, Laca A, García LA, et al. (2001) Controlled malolactic fermentation in cider using *Oenococcus oeni* immobilized in alginate beads and comparison with free cell fermentation. *Enzyme Microb Technol* 28: 35–41.
27. Valles BS, Bedriñana RP, Tascón NF, et al. (2007) Yeast species associated with the spontaneous fermentation of cider. *Food Microbiol* 24: 25–31.
28. Nedovic VA, Durieuxb A, Nedervelde LV, et al. (2000) Continuous cider fermentation with co-immobilized yeast and *Leuconostoc oenos* cells. *Enzyme Microb Technol* 26: 834–839.
29. Joshi VK, Bhardwaj JC (2011) Effect of different cultivars yeasts (free and immobilized cultures) of *S. cerevisiae* and *Schizosaccharomyces pombe* on physico-chemical and sensory quality of plum based wine for sparkling wine production. *Int J Food Ferment Technol* 1: 69–81.
30. Joshi VK, Sharma PC, Attri BL (1991) A note on the deacidification activity of *Schizosaccharomyces pombe* in plum musts of variable composition. *J Appl Microbiol* 70: 386–390.
31. Lee PR, Kho HSC, Yu B, et al. (2013) Yeast ratio is a critical factor for sequential fermentation of papaya wine by *Williopsis saturnus* and *Saccharomyces cerevisiae*. *Microb Biotechnol* 6: 385–393.
32. Varakumar S, Naresh K, Reddy OVS (2012) Preparation of mango wine using a new yeast-mango-peel immobilized biocatalyst system. *Czech J Food Sci* 30: 557–566.
33. Oliveira MES, Pantoj L, Duarte WF, et al. (2011) Fruit wine produced from cagaita (*Eugenia dysenterica* DC) by both free and immobilised yeast cell fermentation. *Food Res Int* 44: 2391–2400.
34. Banat I, Nigam P, Marchant R (1992) Isolation of thermo-tolerant, fermentative yeasts growing at 52 ^{o}C and producing ethanol at 45 ^{o}C and 50 ^{o}C. *World J Microb Biot* 8: 259–263.
35. Singh D, Nigam P, Banat IM, et al. (1998) Ethanol production at elevated temperatures and bioethanol concentrations: Part II—use of *Kluyveromyces marxianus* IMB3. *World J Microb Biot* 14: 823–834.
36. Ganatsios V, Koutinas AA, Bekatorou A, et al (2014) Promotion of maltose fermentation at extremely low temperatures using a cryotolerant *Saccharomyces cerevisiae* strain immobilised on porous cellulosic material. *Enzyme Microb Technol* 66: 56–59.

37. Toit MD, Engelbrecht L, Lerm E, et al. (2011) Lactobacillus: The Next Generation of Malolactic Fermentation Starter Cultures-an Overview. *Food Bioproc Technol* 4: 876–906.
38. Genisheva Z, Mussatto SI, Oliveira JM, et al. (2013) Malolactic fermentation of wines with immobilised lactic acid bacteria—influence of concentration, type of support material and storage conditions. *Food Chem* 138: 1510–1514.
39. Riordan C, Love G, Barron N, et al. (1996) Production of ethanol from sucrose at 45 ^{o}C by alginate-immobilized preparations of the thermotolerant yeast strain *Kluyveromyces marxianus* IMB3. *Bioresource Technol* 55: 171–173.
40. Nigam P, Banat IM, Singh D, et al. (1997) Continuous ethanol production by thermotolerant *Kluyveromyces marxianus* IMB3 immobilized on mineral Kissiris at 45 ^{o}C. *World J Microb Biot* 13: 283–288.
41. Love G, Gough S, Brady D, et al. (1998) Continuous ethanol fermentation at 45 ^{o}C using *Kluyveromyces marxianus* IMB3 immobilized in Calcium alginate and kissiris. *Bioproc Eng* 18: 187–189.
42. Love G, Nigam P, Barron N, et al. (1996) Ethanol production at 45 ^{o}C using preparations of *Kluyveromyces marxianus* IMB3 immobilized in calcium alginate and kissiris. *Bioproc Eng* 15: 275–277.
43. Agouridis N, Bekatorou A, Nigam P, et al. (2005) Malolactic fermentation in wine with *Lactobacillus casei*cells immobilized on delignified cellulosic material. *J Agric Food Chem* 53: 2546–2551.
44. Ciani M (2008) Continuous deacidification of wine by immobilized *Schizosaccharomyces pombe* cells: Evaluation of malic acid degradation rate and analytical profiles. *J Appl Microbiol* 79: 631–634.
45. Singh D, Dahiya J, Nigam P (1995) Simultaneous raw starch hydrolysis and ethanol fermentation by glucoamylase from *Rhizoctonia solani* and *Saccharomyces cerevisiae*. *J Basic Microb* 35: 117–121.
46. Servetas I, Berbegal C, Camacho N, et al. (2013) *Saccharomyces cerevisiae* and *Oenococcus oeni* immobilized in different layers of a cellulose/starch gel composite for simultaneous bioethanolic and malolactic wine fermentations. *Process Biochem* 48: 1279–1284.
47. Verma G, Nigam P, Singh D, et al. (2000) Bioconversion of starch to ethanol in a single-step process by coculture of amylolytic yeasts and Saccharomyces cerevisiae 21. *Bioresource Technol* 72: 261–266.
48. Wati L, Dhamija SS, Singh D, et al. (1996) Characterisation of genetic control of thermotolerance in mutants of *Saccharomyces cerevisiae*. *Genet Eng Biotechnol* 16: 19–26.
49. Volschenk H, Viljoen M, Grobler J, et al. (1997) Engineering pathways for malate degradation in *Saccharomyces cerevisiae*. *Nat Biotechnol* 15: 253–257.
50. Banat I, Singh D, Nigam P, et al. (2000) Potential use of thermo-tolerant fermentative yeasts for industrial ethanol production. *Res Adv Food Sci* 1: 41–55.
51. Song X, Li Y, Wu Y, et al. (2018) Metabolic engineering strategies for improvement of ethanol production in cellulolytic *Saccharomyces cerevisiae*. *FEMS Yeast Res*, 18.
52. Dmytruk KV, Ruchala J, Grabek-Lejko D, et al. (2018) Autophagy-related gene ATG13 is involved in control of xylose alcoholic fermentation in the thermotolerant methylotrophic yeast *Ogataea polymorpha*. *FEMS Yeast Res*, 18.

53. Veselina Petrova (2017) Enerkem launches commercial production of ethanol from waste. Available from: https://renewablesnow.com/news/enerkem-launches-commercial-production-of-ethanol-from-waste-583674/.
54. India's First 2G ETHANOL Production Plant, News in Science, 2016. Available from: https://www.youtube.com/watch?v=0GyAGDqWmjw.
55. Clariant's Cellulosic Ethanol Production Plant, Straubing, Bavaria, 2012. Available from: https://www.chemicals-technology.com/projects/sud-chemie-ethanol/.
56. Nigam PS (2017) An overview: Recycling of solid barley waste generated as a by-production distillery and brewery. *Waste Manage* 62: 255–261.

AIMS Press