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Review

# Fuel ethanol production: Process design trends and integration opportunities

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**Abstract**

Current fuel ethanol research and development deals with process engineering trends for improving biotechnological production of ethanol. In this work, the key role that process design plays during the development of cost-effective technologies is recognized through the analysis of major trends in process synthesis, modeling, simulation and optimization related to ethanol production. Main directions in techno-economical evaluation of fuel ethanol processes are described as well as some prospecting configurations. The most promising alternatives for compensating ethanol production costs by the generation of valuable co-products are analyzed. Opportunities for integration of fuel ethanol production processes and their implications are underlined. Main ways of process intensification through reaction–reaction, reaction–separation and separation–separation processes are analyzed in the case of bioethanol production. Some examples of energy integration during ethanol production are also highlighted. Finally, some concluding considerations on current and future research tendencies in fuel ethanol production regarding process design and integration are presented.


*Keywords:* Fuel ethanol; Process design; Process synthesis; Process integration; Co-products

## 1. Introduction

Ethanol is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the worldwide utilization of fossil fuels. However, the production of ethanol is a complicated process. The transformation of such biological resources as energy-rich crops (like sugar cane or corn) or lignocellulosic biomass requires the conditioning or pretreatment of the feedstocks for fermenting organisms to convert them into ethanol. Then, aqueous solutions of ethanol should be concentrated for obtaining hydrous ethanol. This product has to be dehydrated in order to be utilized as an oxygenate for gasoline, the trade form in which ethanol is mostly employed in the transportation sector. The complexity of this process partly explains why fuel ethanol has not played a leading role in comparison to cheaper oil derived fuels. Only in the last years due to rising environmental concerns and to the periodic crises in some of the larger oil exporting countries, has bioethanol become a viable and realistic alternative in the energy market.

Therefore, the development of cost-effective technologies for fuel ethanol production is a priority for many research centers, universities and private firms, and even for different governments. Due to the large amount of existing and not completely developed technologies for the production of ethanol (especially from lignocellulosic biomass), the application of process engineering tools is required. Process engineering applied to the production of fuel ethanol includes the design of new innovative process configurations aimed at reducing ethanol production costs. Through process design, product diversification for ethanol production processes can be achieved implying the improvement of their costs structure thanks to co-product

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credits. On the other hand, the development of environmentally friendly technologies for bioethanol production can be carried out utilizing different design approaches.

In addition, process synthesis, as a tool of process design, allows the formulation and assessment of many technological flowsheets for finding those ones with improved performance indicators (e.g. techno-economical and environmental indexes). In this way, the impact of specific technologies over the global process and the production costs can be elucidated. Process optimization is another crucial tool employed within the framework of process design. Optimization plays a decisive role not only during the experimentation, but also during the design steps. Particularly, in the case of ethanol produced from lignocellulosic biomass, production costs were reduced during the 80s, but there has been a little drop in the projected costs since 1991 based on the technologies developed for the US Department of Energy. It is believed that some technologies have reached their inherent limits and other less expensive alternatives could replace them. Consequently, advanced models for optimization of ethanol production costs utilizing a systemic approach that permits the identification of opportunities for overcoming the technological barriers should be developed (National Academy of Science, 1999). Other issues related to process engineering as process modeling and simulation underlie the successful design of alternative process configurations for ethanol production. This is particularly true in the case of continuous ethanolic fermentations where dynamic analysis is the key for an appropriate design of such processes.

One of the most important approaches for the design of more intensive and cost-effective process configurations is process integration. Process integration looks for the integration of all operations involved in the production of fuel ethanol. This can be achieved through the development of integrated bioprocesses that combine different steps into one single unit. Thus, reaction–separation integration by removing ethanol from the zone where the biotransformation takes place, offers several opportunities for increasing product yield and consequently reducing product costs. Other forms of integration may significantly decrease energetic costs of specific flowsheet configurations for ethanol production. Process integration is gaining more and more interest due to the advantages related to its application in the case of ethanol production: reduction of energy costs, decrease in the size and number of process units, intensification of the biological and downstream processes.

Very valuable and interesting reviews have been published on the theme of fuel ethanol production especially from lignocellulosic biomass (Chandrakant and Bisaria, 1998; Lee, 1997; Lin and Tanaka, 2006; Lynd, 1996; Wyman, 1994) or covering some key related issues like the cellulose utilization (Béguin and Aubert, 1994; Lynd et al., 2002; Zhang and Lynd, 2004). However, the analysis of integration features of the overall process for fuel ethanol production from different feedstocks has not been the main objective of these reviews. In addition, various process design issues have not always been sufficiently highlighted in order to get a comprehensive picture of the role that process engineering can play for improving fuel ethanol production processes. This paper attempts to achieve this aim. Therefore, the objective of this work is to review the state of the art in bioethanol production from a process engineering point of view, and explore integration as an important avenue for process improvement in the production of this liquid biofuel.

## 2. Trends in process design for fuel ethanol production

The design of cost-effective processes for fuel ethanol production implies the selection of the most appropriate feedstocks, and the selection and definition of a suitable process configuration making possible the conversion of raw materials into the end product meeting given specifications. The task of defining a proper configuration of the process requires the generation and assessment of many process flowsheets for finding those ones with improved performance indicators. This step of process design is called process synthesis. During process analysis, the structure of the synthesized flowsheets is established in order to improve the process through a more detailed insight of it. In both steps, process modeling and simulation play a significant role although with different level of detail (basically, short-cut models for synthesis and rigorous models for analysis). In this work, the importance of the feedstock as a key factor in the economy of ethanol production processes is highlighted. Process synthesis features for bioethanol production are also examined considering different trends utilized for reducing production costs. In addition, some aspects involving process analysis methodologies are stressed in order to show how they may contribute to the design of successful technologies in the case of fuel ethanol production.

### 2.1. Evaluation of feedstocks

Bioethanol can be produced from raw materials containing fermentable sugars as sugar cane and beet that are rich in sucrose. In addition, bioethanol may also be produced from some polysaccharides that can be hydrolyzed for obtaining sugars convertible into ethyl alcohol. Starch contained in grains is the major polymer used for ethanol production. Lignocellulosic biomass (a complex comprised of several polysaccharides) is the most promising feedstock considering its great availability and low cost, but the large-scale commercial production of fuel ethanol from lignocellulosic materials has still not been implemented. For designing fuel ethanol production processes, the assessment of the utilization of different feedstocks (i.e. sucrose containing, starchy materials, lignocellulosic biomass) is required considering the big share of raw materials in ethanol costs. In the present review, some trends in the evaluation of feedstocks for process design in the case of bioethanol production are highlighted below.

*2.1.1. Sucrose-containing feedstocks*

Sugar cane, either in the form of cane juice or cane molasses, is the most important feedstock utilized in tropical and sub-tropical countries for producing ethanol. In European countries, beet molasses are the most utilized sucrose-containing feedstock. Besides these energy crops, sweet sorghum has become a perspective raw material considering that from its stalks can be extracted a juice with a high content of sucrose, its grains contain a high amount of starch and its bagasse is an important source of lignocellulosic biomass (Winner Network, 2002). The conversion of sucrose into ethanol is easier compared to starchy materials and lignocellulosic biomass because previous hydrolysis of the feedstock is not required since this disaccharide can be broken down by the yeast cells; in addition, the conditioning of the cane juice or molasses favors the hydrolysis of sucrose.

In terms of the design of ethanol production processes from sucrose-containing materials, the availability and transport costs of the feedstock continues playing a crucial role when new cost-effective production facilities are being projected despite the relative maturity of the involved conversion technologies. Maiorella et al. (1984) performed a detailed economic analysis of the production of hydrous (azeotropic) ethanol from molasses; their results showed that feedstock costs comprise up to 70% of the final ethanol price for the case of molasses. Nguyen and Prince (1996) have proposed rules for optimization of the size of a plant for ethanol production obtaining a simple relationship between transport costs of feedstock, in this case sugar cane, and the production costs. Likewise, they showed that transport costs per unit of produced ethanol correspond to 0.4–0.6 of the unit production costs for an optimal plant size. Moreover, the use of combined crops of sugar cane and sweet sorghum can reduce the ethanol costs in the conditions of Australia. The possibility of producing fuel ethanol from the juice of sweet sorghum in the conditions of North China has been examined by Gnansounou et al. (2005). These authors recommend the design of flexible plants capable of producing both sugar and ethanol from sweet sorghum juice considering the volatility of sugar market. In addition, the production of ethanol from sweet sorghum bagasse is proposed instead of burning it for power production.

*2.1.2. Starchy materials*

To produce ethanol from starch it is necessary to break down the chains of this carbohydrate for obtaining glucose syrup, which can be converted into ethanol by yeasts. This type of feedstock is the most utilized for ethanol production in North America and Europe. Corn and wheat are mainly employed with these purposes. In tropical countries, other starchy crops as tubers (e.g. cassava) can be used for commercial production of fuel ethanol.

The economic evaluation of different starchy materials for fuel ethanol production at small scale level was dealt in earlier works (Bengtson, 1983; Ganesh and Mowat, 1985; Meo, 1984; Mullis and NeSmith, 1984). An example of the great effect the value of feedstock has on ethanol production costs is provided by Batchelor et al. (1994). According to these authors in the case of the United Kingdom, if the price per ton of wheat is £115, the net costs of ethanol are calculated in £0.38 per liter, but if wheat is cultivated next to the plant and obtained at a price of £45 per ton, ethanol costs can fall down to £0.21 per liter. Tiffany and Eidman (2003) provide a useful spreadsheet model that analyzes the economic factors associated with success or failure of corn dry-milling ethanol plants. For this kind of plants in USA, ethanol, corn and natural gas prices, and ethanol yields can each drastically affect net margins of ethanol plants.

Other example of the importance of feedstock during process design is the work of Zhang et al. (2003) done in China through simulation of fuel ethanol production process from cassava employing a model based on Excel (Microsoft Corp., USA). From this work, it was demonstrated that the production of ethanol as a gasoline oxygenate is economically feasible if the price of cassava is maintained low. In addition, if the yields of cassava crops are augmented and the production of co-products from the process is optimized, ethanol can become a serious competitor of gasoline as a fuel. Moreover, the plantation of 200,000 ha of cassava would have a significant social impact in the province of Guangxi in Southwest China.

*2.1.3. Lignocellulosic biomass*

Currently, a large amount of studies regarding the utilization of lignocellulosic biomass as a feedstock for producing fuel ethanol is being carried out worldwide. For countries where the cultivation of energy crops is difficult, lignocellulosic materials are an attractive option for the production of biofuels. The main challenge in the conversion of biomass into ethanol is the pretreatment step. Due to the structure of the lignocellulosic complex, the pretreatment is required for its degradation, the removal of lignin, the partial or total hydrolysis of the hemicellulose, and the decrease in the fraction of crystalline cellulose related to the amorphous cellulose, the most suitable form for the subsequent hydrolysis step. In this step, the cellulose undergoes enzymatic hydrolysis in order to obtain glucose that is transformed into ethanol by process microorganisms. Eventually, the sugars released during the hydrolysis of hemicellulose can be converted into ethanol as well. Consequently, the involved technologies are more complex leading to higher ethanol production costs compared to cane, beet or corn. However, the fact that many lignocellulosic materials are by-products of agricultural activities, industrial residues or domestic wastes offers huge possibilities for the production of fuel ethanol at large scale as well as its global consumption as a renewable fuel. It is considered that lignocellulosic biomass will become the main feedstock for ethanol production in the near future.

Earlier works have compared the costs of sugars derived from corn with those ones derived from lignocellulosic

biomass as a first stage of the analysis for the possible implementation of biomass-to-ethanol process (Ladisch and Svarczkopf, 1991). Similarly, some economic evaluations of different feedstocks including lignocellulosic materials for fuel ethanol production at small scale level have been reported (Koutinas et al., 1981; Meo, 1984). In the above-cited work of Maiorella et al. (1984), a detailed economic analysis of the production of ethanol from cellulose hydrolyzate considering several technological options was also performed; in this paper, the concentration of cellulose hydrolyzate was proposed for reducing the costs of separation procedures.

The evaluation of biomass costs is a very important issue that is related to its availability in the neighboring areas to the production plants of fuel ethanol. Zhan et al. (2005) indicate that the costs of the feedstock depend on the plant location, plant size and the procedure for acquiring the feedstock. These authors have designed a model based on geographic information system (GIS) for assessing the price policy to acquire feedstock. It was shown that it is more suitable to pay a specific price per ton to the feedstock supplier and then to pay the transport costs of feedstock than to pay a fixed price per ton of feedstock sent by the supplier. The studied feedstock was the switchgrass in the state of Alabama (USA), a fast-growing grass with a high content of lignocellulosics. In the same way, these authors evaluated the best location for a bioethanol production facility from mentioned feedstock (Noon et al., 2002). Kumar et al. (2005) have proposed an interesting way for integrating the feedstock transport to the ethanol production facility and the saccharification process named simultaneous transport and saccharification. These authors consider that the enzymatic hydrolysis of corn stover can be carried out in pipelines during its transport; the hydrolyzed corn stover could directly enter the ethanol fermentation plant, saving about 0.2 US cents/L EtOH.

Based on literature data, Murphy and McCarthy (2005) performed the economic analysis for the production of fuel ethanol from energy crops (sugar beet) and lignocellulosic biomass (waste paper) and showed the importance of the economies of scale and choice of feedstock. Best results were obtained for the production of 200 million liters per year of ethanol from waste paper attaining greenhouse-gas savings that would equate to 18% of the 1990 transport emissions under the conditions of Ireland. Bridgewater and Double (1991) reported a model for the calculation of production costs of liquid fuels from renewable resources (lignocellulosic biomass, wheat, sugar beet) by thermochemical and biochemical processes including ethanol, methanol, gasoline and diesel. Lynd et al. (1999) provide new metrics showing the favorable influence of co-products produced from non-carbohydrate portions of feedstocks on the definition of the effective cost of carbohydrate for biological processing. These metrics evidence that the conversion processes for corn wet-milling technology are developed to the point that the price of feedstock represents the largest share of total product value, and that processing cost margins are relatively small. That is not the case of lignocellulosic biomass that demonstrates the relative immaturity of the technology for its conversion.

### 2.2. Process synthesis for conversion of lignocellulosic biomass to ethanol

Process synthesis is oriented to the generation of different process configurations (flowsheets) that could become viable alternatives for the production of a given product. During the generation of flowsheets, the different relationships among the diverse unit operations and processes corresponding to a particular configuration are considered in order to organize the material and energy flows. This generation process is mainly based on decomposition procedures of the overall system and heuristic rules. Other process synthesis approaches rely on the fact that the proposal of new flowsheets can be accomplished by analyzing the process at a more fundamental level, e.g. as a combination of transport processes or thermodynamic phenomena, or using an optimization-based methodology (Li and Kraslawski, 2004; Westerberg, 2004). Considering that the biomass-to-ethanol conversion technologies are relatively immature and are not completely developed compared to cane ethanol or ethanol from starch, process synthesis methodologies can offer invaluable tools for the design of more cost-effective configurations with improved techno-economic and environmental indicators. Therefore, in this work, some trends in the process synthesis for fuel ethanol production are presented emphasizing the proposal and development of different process flowsheets, and the utilization of simulation tools for this task.

Overall fuel ethanol production from lignocellulosic biomass includes five main steps: biomass pretreatment, cellulose hydrolysis, fermentation of hexoses, separation and effluent treatment (see Fig. 1). Furthermore, detoxification and fermentation of pentoses released during the pretreatment step can be carried out. The sequential configuration employed to obtain cellulosic ethanol implies that the solid fraction of pretreated lignocellulosic material undergoes hydrolysis (saccharification); this fraction contains the cellulose in an accessible to acids or enzymes form. Once hydrolysis is completed, the resulting cellulose hydrolyzate is fermented and converted into ethanol. This process is called separate hydrolysis and fermentation (SHF). SHF is one of the configurations that has been tested more extensively. Pentose fermentation, when it is carried out, is accomplished in an independent unit. The need of separate fermentations is due to the fact that pentose utilizing microorganisms ferment pentoses and hexoses slower than microorganisms that only assimilate hexoses. Moreover, the former microorganisms are more sensitive to the inhibitors and to the produced ethanol; for this reason, the hemicellulose hydrolyzate resulting from pretreatment should be detoxified. If the fermentation of the hemicellulose and cellulose hydrolyzates is carried out in a

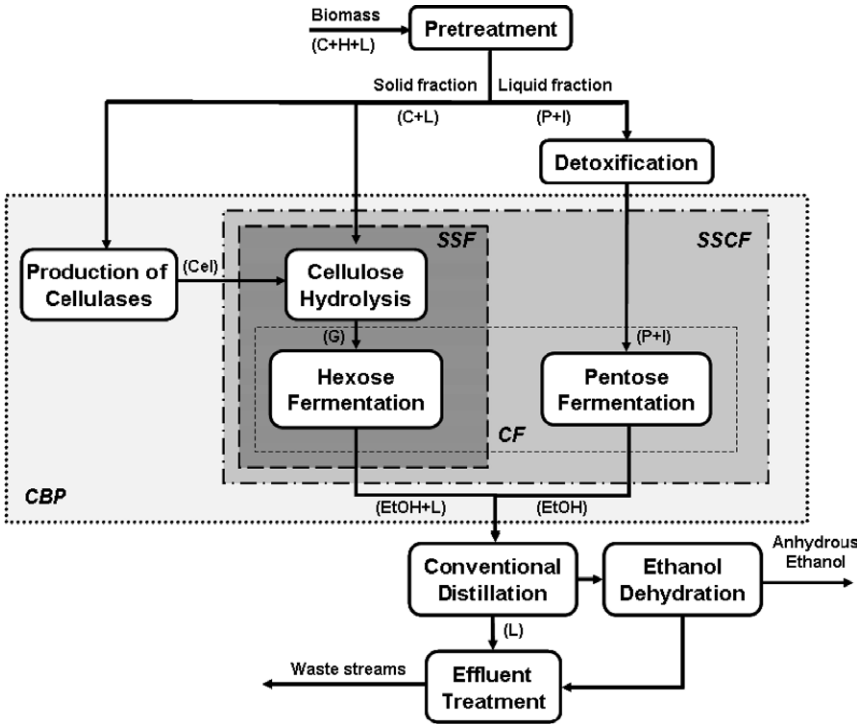


Fig. 1. Generic block diagram of fuel ethanol production from lignocellulosic biomass. Possibilities for reaction–reaction integration are shown inside the shaded boxes: CF, co-fermentation; SSF, simultaneous saccharification and fermentation; SSCF, simultaneous saccharification and co-fermentation; CBP, consolidated bioprocessing. Main stream components: C, cellulose; H, hemicellulose; L, lignin; Cel, cellulases; G, glucose; P, pentoses; I, inhibitors; EtOH, ethanol.

separate way, less liquid volumes of hydrolyzate have to be detoxified (Olsson and Hahn-Hägerdal, 1996). In a later research utilizing lignocellulosic materials that are hard to degrade as softwood (mainly spruce), Tengborg et al. (2001) demonstrated that the liquid fraction of the steam-exploded biomass inhibits the enzymatic hydrolysis. Thus, these authors proposed the fermentation of this fraction before the hydrolysis resulting in the neutralization of this inhibitory effect. It was shown that neither furfural nor hydroximethylfurfural have inhibitory effects on this process; aliphatic acids, furan derivatives or phenolic compounds appear to be the inhibitory substances. These compounds are generated during the pretreatment step.

### 2.2.1. Recycling possibilities

In the search of improved process flowsheets, recycling of some streams have been proposed in order to attain a more complete utilization of sugars formed within the process or for reusing expensive bioagents like cellulolytic enzymes. The recycling of pentoses formed during the hydrolysis of the hemicellulose was studied by Galbe and Zacchi (1994) resulting in the increase of ethanol yield and the decrease of energy consumption. However, these flowsheets have the inconvenient that the concentration of fermentation inhibitors is augmented. For this reason, further research for finding resistant microorganisms or the best way of detoxification should be carried out including the utilization of cellulase-producing fungi like *Trichoderma reesei* (Palmqvist et al., 1997).

Lee et al. (1995) have also proposed the reutilization of cellulases through different strategies of recycling using the residual substrates remaining during the batch hydrolysis of cellulose, although the content of lignin in the substrate negatively affected the cellulase activity. Earlier, Mes-Hartree et al. (1987) had proposed the recycling of both cellulases and substrate in order to save enzymes and to utilize the residual substrate for producing cellulases for the same process. Nguyen et al. (1999) propose the use of both microfiltration and ultrafiltration in order to collect the cellulases from the enzymatic hydrolysis reactor during ethanol production from municipal solid waste (MSW). The configuration corresponds to SHF process and the hydrolysis reactor works in a fed-batch regime. The authors claim that this technique for cellulase recycling combined with fed-batch operation allows significant reductions in the cost of cellulose hydrolysis. However, the difficulties related to the recycling of adsorbed cellulases in the case of continuous processes, the increase in the expected effectiveness of cellulolytic enzymes, among other factors, have narrowed the application of this technique to batch and fed-batch SHF processes.

With the help of techno-economical models based on the SHF process, the recycling of cellulases has been proposed during the enzymatic treatment of the wood as well as the duplication of hydrolysis time (from 24 to 48 h). In this way, a 27% reduction in the ethanol production costs from hardwood (oak) and 38% reduction from softwood (conifers) were estimated. It is pointed out that this technology

is in its infancy and that the processes are far away of its optimal operation (Gregg et al., 1998).

*2.2.2. Process flowsheet development for biomass-to-ethanol conversion*

The conformation of a network for the study of biomass-to-ethanol conversion named "Biotechnology for the Conversion of Lignocellulosics" under the International Energy Agency with the participation of research groups from USA, Canada and Sweden has been reported (Saddler, 1992). To this aim, a generic process from wood has been designed (Gregg and Saddler, 1995). Since that time, it was evident that the interactions among the different operations should be considered in terms of process design and optimization. This is valid for all feedstocks used for ethanol production. In these early works, environmental aspects were taken into account as constraints and not as objectives of the process. Other early works dealing with process synthesis for the production of fuel ethanol from biomass were oriented to the estimation of production costs of ethanol from wood chips and to the analysis of the interdependence of process parameters. In particular, the following parameters were defined as the most significant: wood cost, enzyme costs, efficiency of the cellulose hydrolysis, ethanol yield from pentoses, efficiency of fractionation process, and the selling price of the by-product lignin (Nguyen and Saddler, 1991).

The significant variety of pretreatment methods of biomass has led to the development of many flowsheet options for ethanol production. Von Sivers and Zacchi (1995) analyzed three pretreatment process for the ethanol production from pine: concentrated acid hydrolysis, two-stage hydrolysis by steam explosion using $SO_2$ and dilute acid, and steam explosion using $SO_2$ followed by the enzymatic hydrolysis. Through sensitivity analysis, these authors showed that none of the processes could be discarded as the less rentable. Using commercial process simulators like Aspen Plus (Aspen Technologies, Inc., USA), this group of authors have evaluated different modifications of the ethanol production process from willow wood employing separate fermentations of hexoses and pentoses. Milling has been suggested as unique pretreatment method before the cellulose hydrolysis since the required equipment are less expensive than the equipment needed for other pretreatment methods like steam explosion or ammonia fiber explosion (AFEX) process, which can account for 6–20% of capital costs of the process. In contrast, milling equipment accounts for about 1% of these costs. However, it is considered that milling has elevated energetic costs. Alvo and Belkacemi (1997) point out that milling of perennial grasses require much less energy that milling of wood. These authors consider that milling as sole pretreatment method should not be discarded as an option taking into account the advantages of this configuration: toxic products of degradation are not formed, soluble carbohydrates of the initial biomass are not destroyed and many rural communities can acquire in an easier way mills in comparison with other expensive pretreatment equipments. This alternative should be evaluated in depth utilizing simulation and optimization tools in the design step.

In USA, the production of ethanol from lignocellulosic biomass is being studied intensively. Ingram et al. (1999) from the University of Florida have carried out significant research on the development of recombinant strains of enteric bacteria for using them during the biomass-to-ethanol process. Current technology implies the use of genetically engineered *Escherichia coli* strain with the natural ability of assimilating both pentoses and hexoses found in the liquid fraction resulting from the dilute-acid pretreatment of lignocellulosic biomass; the main *Zymomonas mobilis* genes encoding the ability for the homofermentative production of ethanol have been integrated into the bacterial chromosome in this strain. The solid fraction from this pretreatment that contains cellulose and lignin undergoes simultaneous saccharification and fermentation (SSF, see Section 4.2) utilizing a recombinant strain of *Klebsiella oxytoca* with the ability to ferment cellobiose and cellotriose, eliminating the need of supplementing *T. reesei* cellulases with β-glucosidase; this strain also has the genes encoding the production of ethanol. The proposed overall process can be observed in Fig. 2a. At present, research efforts are being oriented to the development of a single microorganism capable of efficiently fermenting both hemicellulosic and cellulosic substrates that will make possible the development of the direct conversion of biomass into ethanol.

The model process designed by the US National Renewable Energy Laboratory (NREL) comprises a previous hydrolysis of wood with dilute acid followed by a simultaneous saccharification and co-fermentation (SSCF, see Section 4.3) process utilizing cellulases produced *in situ* by genetically engineered *Z. mobilis* with the ability of transforming both glucose and xylose into ethanol (see Fig. 2b). The process is energetically integrated using the heat generated during the combustion of methane formed in the anaerobic treatment of wastewater from pretreatment and distillation steps (Wooley et al., 1999a). In addition, the burning of lignin allows the production of energy for the process and a surplus in form of electricity. The production of one liter of ethanol by this process is calculated US$0.396, whereas the ethanol production cost from corn is US$0.232 (McAloon et al., 2000). A pilot plant designed for the conversion of lignocellulosic biomass into ethanol was built and operated with the aim of supporting industrial partners for the research and development of biomass ethanol technology (Nguyen et al., 1996). In this plant, tests in continuous regime for the utilization of lignocellulosic residues of low cost and great availability like corn fiber were carried out (Schell et al., 2004). The objective of these tests consisted in the assessment of the operation of the integrated equipments and in the generation of data on the process performance. This type of plants allows the acquisition of valuable experience considering the future implementation of the industrial process, as well as the

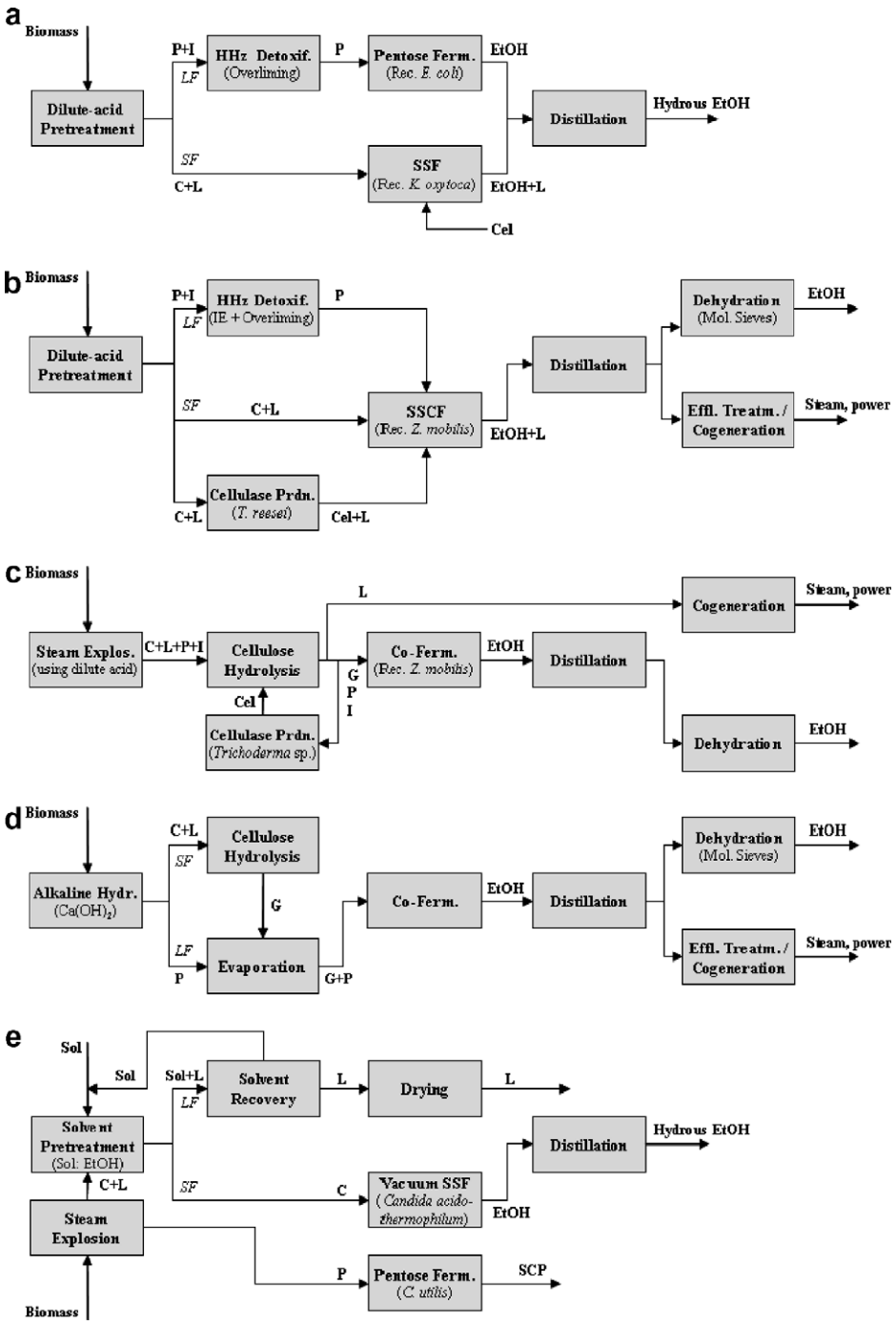


Fig. 2. Some proposed flowsheet configurations for ethanol production from lignocellulosic biomass. (a) Process based on utilization of enteric bacteria (Ingram et al., 1999). (b) NREL model process (Wooley et al., 1999a). (c) Iogen's process (Tolan, 2002). (d) Process proposed by Reith et al. (2002). (e) IIT Delhi process (Ghosh and Ghose, 2003). Main stream components: C, cellulose; L, lignin; G, glucose; P, pentoses; I, inhibitors; Cel, cellulases; EtOH, ethanol; Sol, solvent; SCP, single cell protein; LF, liquid fraction; SF, solid fraction; HHZ, hemicellulose hydrolyzate; Rec, recombinant; IE, ion exchange.

feedback of the models utilized during the design step. In addition, feasibility studies carried out by NREL help industrial partners in making decisions about the potential implementation of these technologies for fuel ethanol production (Kadam et al., 2000; Mielenz, 1997). Future trends for costs reduction in the case of NREL process include more efficient pretreatment of biomass, improvement of specific activity and productivity of cellulases, the possibility of carrying out SSCF process at higher temperatures, improvement of recombinant microorganisms for a greater assimilation of all the sugars released during the pretreatment and hydrolysis processes, and further development of co-generation system. Nagle et al. (1999) proposed an alternative configuration that involves a total hydrolysis of yellow poplar using a three-stage countercurrent dilute-acid process validated at experimental level.

The obtained hydrolyzate is co-fermented by the recombinant strain of *Z. mobilis*. In this case, the lignin is recovered prior the fermentation. Aspen Plus was utilized for generating the needed information in order to evaluate the economic performance of the whole flowsheet configuration through a spreadsheet model. Optimized values of the key process variables obtained from the simulation are utilized as target values for bench-scale research in order to design an advanced two-stage engineering-scale reactor for a dilute-acid hydrolysis process.

Iogen Corporation, a major manufacturer of industrial enzymes in Canada, developed a SHF process comprising a dilute-acid-catalyzed steam explosion and the removal of the major part of the acetic acid released during the pretreatment, the use of *Saccharomyces cerevisiae* as a fermenting organism, distillation of broth, ethanol dehydration and disposal of stillage in landfill (Tolan, 2002). Later modifications involve the co-fermentation of both hexoses and pentoses using genetically modified strains of microorganisms like yeasts or bacteria (Fig. 2c). Using the recombinant *Z. mobilis* strain patented by NREL, Lawford and Rousseau (2003) tested two configurations for ethanol production using the conceptual design based on SHF developed by Iogen. These authors demonstrated that a configuration involving the continuous pentose fermentation using the recombinant *Z. mobilis* strain, and the separate enzymatic hydrolysis followed by continuous glucose fermentation using a wild type strain of *Z. mobilis* is the most appropriate in comparison to the use of the co-fermentation process after the enzymatic hydrolysis or the use of an industrial yeast strain during the glucose fermentation.

Reith et al. (2002) have reviewed different processes for production of biomass ethanol and concluded that verge grass, willow tops and wheat milling residues could be potential feedstock for fuel ethanol production in the conditions of the Netherlands. These authors constructed a model using Excel for the system description of generic biomass-to-ethanol process. This process involves the evaporation of the stream from saccharification step in such a way that the sugar concentration allows a final ethanol concentration of at least 8.5 vol.% in the fermentation broth. In addition, pretreatment using $Ca(OH)_2$ was included in the analysis (see Fig. 2d). The advantage of using this type of pretreatment is that inhibitors are not formed implying that detoxification step is not necessary. The evaluation showed that currently available industrial cellulases accounts for 36–45% of ethanol production costs, and therefore, a 10-fold reduction in the cellulase costs and a 30% reduction in capital costs are required in order to reach ethanol production costs competitive with starch ethanol. These evaluation approaches indicate the need for developing processes that contribute to improve one or all of the four critical areas related to cellulase research: increased thermostability, improved cellulose binding, increased specific activity, and reduced non-specific binding to lignin (Mielenz, 2001). The thermal stability of cellulases should be improved considering that the increase of temperature at which these enzymes can operate implies a significant increase in the cellulose hydrolysis rate. For this, research in protein engineering can give the key for undertaking this challenging issue (Wooley et al., 1999b). Sheehan and Himmel point out that a 10-fold increase in enzyme specific activity could lead to production costs savings of more than 15.85 US cents per liter of ethanol. These authors also mention the possible strategies for increasing the specific activity of cellulases: synergism of cellulases from different microbial sources, enhancement of the efficiency of active sites by protein engineering or by random mutagenesis, increase in thermal tolerance, enhancement of cellulose decrystallization, and reduction of non-specific binding.

Ghosh and Ghose (2003) report the model process for bioethanol production proposed by Indian Institute of Technology (IIT) in Delhi (India). This process involves two pretreatment steps: steam explosion for xylose production followed by solvent pretreatment for delignification of biomass. The released pentoses are utilized for single cell protein production, whereas the cellulose undergoes simultaneous saccharification and fermentation. The SSF reactor is coupled with vacuum cycling and has a stepwise feeding of cellulose (see Fig. 2e). The process has been tested in pilot plant using rice straw as a feedstock. However, the obtained product is hydrous ethanol (95% v/v) and the production costs (US$0.544/L) are higher than those expected for the production of dehydrated ethanol through the NREL model process (US$0.395). The consideration of adsorption separation stage (instead distillation) increases the cost of ethanol by about 50%. The possibility of using alkali pretreatment was also assessed, but the costs increase due to lower by-product credits (low quality of obtained lignin as a fuel).

Pan et al. (2005) from the University of British Columbia and Lignol Innovations (Canada) report the preliminary evaluation of the so-called Lignol process for processing softwoods into ethanol and co-products. This process makes use of the organosolv process for obtaining high quality lignin allowing the fractionation of the biomass prior to the main fermentation. For this, the process utilizes a blend of ethanol and water at about 200 °C and 400 psi. For ethanol production, SHF or SSF has been tested. Streams containing hemicellulose sugars, acetic acid, furfural and low molecular weight lignin are also considered as a source of valuable co-products. Until now, the Lignol process has only been operated in a three-stage batch mode but simulations studies indicate an improved process economics by operating the plant in continuous mode (Arato et al., 2005). Gong et al. (1999) report a fractionation process employing corn cob and aspen wood chips as feedstocks and utilizing alkaline pretreatment with ammonia that favors the separation of lignin and extractives. After this step, the hemicellulose is hydrolyzed with dilute acid and releasing sugars are fermented by xylose-assimilating yeast; finally the cellulose is converted into

ethanol by batch SSF using a thermotolerant yeast strain. So and Brown (1999) performed the economic analysis of the Waterloo Fast Pyrolysis process comprising a 5% acid pretreatment, fast pyrolysis, levoglucosan hydrolysis and the use of two cultures, *S. cerevisiae* and *Pichia stipitis*, to ferment hexoses and pentoses, respectively. These authors also analyzed the SSF process of dilute-acid pretreated feedstock that comprises the pentose fermentation by recombinant *E. coli* for xylose fermentation, and the SHF process of dilute-acid pretreated feedstock using a strain of *Candida shehatae* for hexose and pentose fermentation. The evaluation indicates that the cost of the fast pyrolysis process is comparable to the other two processes in terms of capital costs, operating costs, and overall ethanol production costs.

Due to the high costs of the feedstocks accounting for more than 20% in the case of the lignocellulosic biomass (Kaylen et al., 2000), the optimization of cellulose conversion is of great importance, especially if it goes with an appropriate handling and utilization of all process streams. Although many related works can be found, the tendency is the optimization of separate process units. This implies that the integration of such separately studied and optimized at different scales units does not always provide the correct information on the global process. This situation is particularly important in the case of the integration of pretreatment step with the biological tansformations. De Bari et al. (2002) undertake this problem emphasizing the scale-up features and the potential of produced by-products for the case of steam-exploded aspen wood chips. The pretreatment step was carried out in a continuous steam explosion pilot plant fed with 0.15 ton/h of dry matter that was coupled with the extraction step in order to separate the lignin and the hemicellulose and carry out the detoxification. The subsequent conversion to ethanol was made by SSF. The process was completed with a packed distillation column with a maximum reboiler capacity of 150 L working batchwise. The experimentation allowed the definition of the best combinations of operation parameters, the selection of the best detoxification procedure, the determination of yields and operation conditions of SSF, the analysis of the distillation for its conversion to hydrogen or ethanol, and the determination of the COD of liquid stream from distillation step. However, this work does not report if any analysis of the process was carried out from the viewpoint of thermodynamic and kinetics fundamentals of the studied system, or if process synthesis procedures were used for the definition of the selected configuration of the process. These tools may contribute to predict the behavior of experimental systems. Similarly, pilot-plant data can provide feedback to the mathematical models used for the analysis of the system, as well as for the study of its stability and operability. In this point, the complementation with simulation tools is invaluable.

In general, it is considered that reductions in processing or conversion costs of lignocellulosic biomass offer the greatest potential for making biobased products like ethanol competitive in the market place in comparison to oil-based products for which high raw material costs are characteristic (National Research Council cited by Dale, 1999). Therefore, the fundamental research on the development of cost-effective processes for biomass processing represents the key for attaining the mentioned competitiveness. Lynd et al. (1999) argue that oil refineries are unlikely to have significant economies of scale advantages in comparison to the expected mature biomass refineries. In this way, the challenges associated with the biomass conversion are related to the recalcitrance of cellulosic biomass (conversion into reactive products like fermentable sugars), and to the product diversification (conversion of reactive intermediates into valuable products).

### 2.2.3. Simulation and optimization approaches

Process synthesis procedures can be significantly enhanced using process simulation packages. These simulators have allowed analyzing several technological options and gaining insight about the process improvements. Cardona and Sánchez (2004, 2006) simulated several technological configurations for the production of fuel ethanol from biomass considering variations in the pretreatment, cellulose hydrolysis, fermentation, separation and effluent treatment steps and taking into account the integration possibilities. The simulations were performed using mostly Aspen Plus. Energy expenditures were utilized for the analysis and comparison of the proposed flowsheets. The obtained results showed that if limitations of the existing technologies are not taken into account, the most appropriate flowsheet should include a dilute-acid pretreatment, SSCF, distillation coupled with pervaporation, and evaporation and recycling of part of the wastewater to be utilized as process water. The need of considering the effect of inhibitors in the co-fermentation process and the degree of streams recycling is highlighted according to the performed sensitivity analysis. Simulation packages are essential for evaluating the amount of studied alternative process flowsheets. Described approach corresponds to knowledge-based process synthesis.

Although some authors have developed own cost evaluation modes (Kaylen et al., 2000; Nguyen and Saddler, 1991), commercial process simulators allow in a more generic and available way the visualization of the interdependences among process units, the same as the effect that different options have over production costs, including the degree with which specific improvements influence on the global process performance. The NREL funds research projects on the production of ethanol from biomass. For defining which projects should be supported among the great amount of proposed improvements to the process, NREL utilizes a model based on Aspen Plus and Excel. This model allows the definition of the most promising research directions through simulation and process analysis of the best proposals aimed at reducing ethanol production costs using lignocellulosic feedstocks (Wooley and Ibsen, 2000). For the simulation with Aspen Plus,

a database of the physical–chemical properties of the main compounds involved during fuel ethanol production from wood chips has been structured (Wooley and Putsche, 1996). With all these elements along with the experience gained in the pilot plant runs, NREL has developed an exhaustive model for the design and costing of biomass-to-ethanol process (Wooley et al., 1999a). The motivation for such a model is the demand for greater reliability and credibility in predicting the costs of bioethanol production considering the increasing demand for cost competitiveness. In addition, the understanding of the economic impacts of proposed research strategies allows guiding the process development (Wooley et al., 1999b). This model is completed with capital cost estimations obtained from vendor quotes and using Icarus cost estimation software. Based on this rigorous developed model of the entire process, Kemppainen and Shonnard (2005) have carried out a comparative life cycle assessment (LCA) for ethanol production from two different feedstocks: virgin timber resources and recycled newsprint from an urban area.

The other main approach is the optimization-based process synthesis that relies on the use of optimization for identifying the best configuration; for this, the definition of a superstructure, which considers a significant amount of variations in the topology of technological configurations of a given process, is required. The evaluation and definition of the best technological flowsheet are carried out through tools like mixed-integer non-linear programming (MINLP). The great advantage of the optimization-based approach is that it allows the generation of a general framework for solving a great variety of process synthesis tasks carrying out a more rigorous analysis of the structure of the process. On the other hand, the main drawbacks are the difficulties in the definition of the superstructure of technological configurations, the mathematical complexity, and the fact that the optimal configuration only can be found within the alternatives considered in the formulated superstructure (Li and Kraslawski, 2004). Sánchez et al. (2006b) have preliminary applied this approach to the production of fuel ethanol from lignocellulosic biomass. To this end, the *Jacaranda* system, an object oriented framework for automated design (Fraga et al., 2000; Steffens et al., 1999), was applied to the biological transformation and separation stages. The software automatically performed the search of the most favorable process alternatives. The results obtained demonstrated that highly integrated configurations (as SSCF) allows the reduction in the ethanol costs. Undoubtedly, the development of this approach will make possible the synthesis of technological flowsheets considering the structure of the system on a mathematical programming basis. The complementation with tools of knowledge-based approach will allow gaining a deeper insight of the overall process needed for the synthesis of technological configurations with increased performance.

### *2.3. Integration of different production lines for ethanol production*

Production lines obtaining ethanol from specific feedstocks can be integrated with other lines in order to take advantage of the potential synergies. Gulati et al. (1996) emphasize integration possibilities of plants producing ethanol from corn in the USA with ethanol production facilities of ethanol from corn fiber (a lignocellulosic residue). These authors consider that if 80% efficiency in the hydrolysis and hexose fermentation and 70% efficiency in the pentose fermentation were assumed, an increase in ethanol yield of 0.033 L/kg of corn could be obtained. Given the great amount of corn processed to ethanol in USA, the development of the technology for ethanol production from corn fiber would offer important benefits to the industry reducing the ethanol costs.

Comparable integration possibilities could be implemented in the case of ethanol production process from wheat. For example, the wheat bran obtained during the milling of wheat can be pretreated and hydrolyzed with acids in order to obtain fermentable sugars for ethanol production, complementing the use of the starch contained in wheat grains (Chotěborská et al., 2004; Palmarola-Adrados et al., 2005). Similarly, the polysaccharide-rich waste stream of a combined wheat starch and ethanol factory, the so-called wheat starch fiber, was tested with amylases and high dosages of cellulases for obtaining an increased concentration of fermentable sugars for ethanol production (Palmarola-Adrados et al., 2004). On the other hand, the treatment of the thin stillage of the process from wheat using preparates containing hemicellulases and other residual enzymatic activities has been proposed (Sørensen et al., 2005). This residue of ethanol process from wheat is rich in arabinoxylan, since its enzymatic treatment allows the production of arabinose and xylose, which can be converted into ethanol or other value added products.

Koutinas et al. (2004) propose a new approach for the production of the culture medium needed for fermentation not only for the biosynthesis of ethanol, but for other biological products. This approach implies a complete restructuring of the cereal bioconversion into a generic microbial feedstock. Fort this, part of the feedstock (whole wheat flour) is sterilized and fermented by the enzyme-producing *Aspergillus awamori* that produces the needed amylases, proteases and other enzymes for the degradation of the major polymers of the wheat kernel, whereas the other part is utilized in the production line for gluten separation. The enzyme-rich filtrate from culture broth is used for the hydrolysis of starch and other polymers contained in the gluten-free flour fraction; the fungal cells contained in the solid residue are autolyzed for obtaining nitrogen compounds. In this way, the unification of these two streams allows the production of a microbial feedstock that can be integrated in an existing fermentation plant.

The feasibility of the integration of an ethanol production facility from softwood forest thinnings to an existing

biomass power plant has been carried out for the conditions of Northern California in USA (Kadam et al., 2000). Obtained results show that a plant processing 800 dry tonnes per day of feedstock could be promising considering an internal rate of return (IRR) of 24% using 25% equity financing, and that the utilization of the biomass residue contributes to the reduction of fire danger.

Grassi (1999) conceptually proposed the integration of the process for ethanol production with the production of methanol. In this integration schema, the $CO_2$ produced during the ethanolic fermentation of sugar solutions could be catalytically hydrogenated to methyl alcohol. For a plant with a capacity of 50,000 ton/year of bioethanol and 35,000 ton/year of biomethanol (total inversion of 35 mill US dollars), the production costs of methanol could reach approximately US$192/ton according to his estimations. The most promising feedstock for this process in the case of tropical or South European countries would be sweet sorghum, while for North European countries, lignocellulosic biomass (mainly wood or herbaceous crops like miscanthus) would be the most appropriate. However, the analysis and simulation of such a process was not reported.

### 2.4. Process analysis tools

#### 2.4.1. Process simulation

A great amount of studies carried out at laboratory scale for the improvement of bioethanol production processes have been simulated in a preliminary way in order to evaluate not only their performance in terms of mass and energy balances, but also their operation and capital costs generated during their possible implementation at industrial level. The quality of the simulation depends on the suitability of models describing the different steps of the process.

Lin and Tanaka (2006) reviewed the classical generic models for the description of ethanol fermentation. These authors point out that unstructured models are frequently used during the routine control of fermentation processes whereas structured models should be used for optimization and control of ethanol fermentation. The main limitation of these models is that they do not considerate simultaneously the four factors affecting ethanol concentration (substrate limitation, substrate inhibition, product inhibition, and cell death). Most of the models reviewed by these authors are related to the simple ethanol fermentation, but it is necessary the analysis of other models applied to more complex processes like co-fermentation, SSF and SSCF. For instance, the model developed by Leksawasdi et al. (2001) considers three of the main factors affecting ethanol concentration during co-fermentation of glucose and xylose. In the case of starch-to-ethanol process, the model of Montesinos and Navarro (2000) for the SSF of wheat flour can be mentioned, although no expressions for cell formation were included.

Many of the proposed models provide the mathematical description of specific configurations of bioreactors and their cultivation regimes for ethanol production. Gilson and Thomas (1995) developed a model for a fluidized-bed reactor with yeast cells immobilized on alginate beads. It was shown that the observed reduction in ethanol yield compared to free yeast cells was caused by substrate restrictions inside the beads and not but changes in the metabolic rate of the immobilized cells. Borzani (1987) derived a Monod-based model for evaluating the maximum value of the mash feeding rate to be used in order to have a completely fermented medium during the fed-batch fermentation of molasses for ethanol production. Converti et al. (2003) provide a simplified modeling of the kinetics of fed-batch fermentation of sugar cane molasses that allows the prediction and control of the performance of this regime of cultivation. Certainly, this tool is very useful for the simulation of the entire process. Tsuji et al. (1986) evaluated the performance of the continuous alcoholic fermentation using a vector-valued objective function. This analysis considered the trade-off among three criteria (ethanol productivity, the ethanol concentration in the broth and the substrate conversion) on the basis of the non-inferior set defined in a vector-valued function space. Costa et al. (2001) used an intrinsic model that took into account cell volume fraction and the dependence of kinetic constants on temperature for continuous vacuum fermentation using yeasts. With the help of this model, the process was optimized using response surface analysis that allowed the determination of operational conditions maximizing high yield and productivity. In the same way, dynamic simulation was performed using the concepts of factorial design in order to determine the best control structures for the process. Maia and Nelson (1993) presented a model of gravitational sedimentation intended to describe the recycling of cells to the bioreactor using a parallel plate sedimenter during continuous fermentation. This model allowed the optimization of the operating conditions in order to efficiently recycle yeasts at high cell density.

Modeling of other process steps of biomass-to-ethanol process has been carried out. This is the case of cellulase production by *T. reesei* using kinetic and neural networks approaches (Tholudur et al., 1999) that allow the optimization of operating conditions for two performance indexes based on the estimated protein value and on the volumetric productivity. An interesting approximation for the determination of the thermodynamic parameters allowing the modeling of vapor–liquid equilibrium of such materials as sugar cane juice or beet molasses was employed by Abderafi and Bounahmidi (1999). This kind of models could permit a more reliable simulation of ethanol production process when commercial simulators are used.

The formulation of proper models for the description of biological processes having place during ethanol production is a crucial issue that has strong influence on the quality of subsequent simulation in a chemical process simulator. Pascal et al. (1995) highlighted these aspects

and illustrated the application of a general kinetic model of alcoholic fermentation during the simulation of overall process in both continuous and fed-batch regimes using Prosim and Prosim Batch (Prosim S.A., France) simulators. The performed simulation was applied to an existing industrial plant producing ethanol from beet molasses by yeasts. The obtained results allowed successfully simulating the operation of the plant providing valuable knowledge for further process optimization. Nevertheless, the separation process was not rigorously simulated and ethanol dehydration step was not taken into account in this case. Naser and Fournier (1988) utilized the commercial package CHEMCAD (Chemstations, Inc., USA) for simulating a plant configuration where extractive fermentation of molasses is carried out in a hollow-fiber membrane extractive fermentor (HFMEF). For this, the model of the reactor was introduced into CHEMCAD as a user added Fortran subroutine. The output of the simulation was read into a spreadsheet handling the economic evaluation of the whole process. Obtained results showed that considerably higher productivity of HFMEF is overshadowed by the present high cost of hollow fibers. However, a reduction of 1.8 US cents/L of ethanol can be achieved for every US$1/sq ft drop in the price of hollow fibers. These authors highlighted that an advance in hollow fiber manufacturing technology could reduce the cost of producing ethanol from molasses by the HFMEF process by up to 20% in comparison to the conventional fermentation process.

The process from corn starch has been simulated as well. From a base case where a traditional liquefaction–saccharification–fermentation process is utilized using yeasts, Krishnan et al. (2000) have proposed substantial modifications partially proven in pilot plant. These modifications include a column packed with immobilized glucoamylase and the use of a fluidized-bed reactor with cells of *Z. mobilis* immobilized in κ-carrageenan gel beads. The performed economic analysis was made with the help of Aspen Plus for the simulation of both processes obtaining results showing that savings in production costs of up to 0.824 US cents per liter of produced ethanol could be achieved using the fluidized-bed reactor. This process should be optimized in order to demonstrate the potential of the synthesized integrated configuration.

Cardona et al. (2005) employed commercial simulators, besides other tools, for the evaluation of capital and operation costs for different types of feedstocks that require diverse processing technologies (lignocellulosic and starchy materials). The results indicate lesser costs for the process from starch in comparison to lignocellulosic biomass due to the greater complexity of biomass processing and considering that this process is not fully developed. Hamelinck et al. (2005) performed a comprehensive evaluation of the biomass-to-ethanol process taking into account three stages of technical development (short-, mid-, and long-term), using different tools as spreadsheets and Aspen Plus for selected process steps. For short-term (5 years), the process involved currently available technologies as dilute-acid pretreatment and SSF; its evaluation shows a total energy efficiency of the feedstock to ethanol (including the generation of electricity) of 38% and ethanol production costs of 22 €/GJ. Mid-term process (10–15 years) was evaluated considering steam explosion pretreatment that enables a better cellulose hydrolysis and a much smaller gypsum waste stream, and SSCF; in this case, a total energy efficiency of 67% and ethanol production costs of 13 €/GJ can be reached considering the utilization of a Biomass-Integrated-Gasifier/Combined Cycle (BIG/CC) for cogeneration of steam and electricity, which represents a more advanced technology than conventional boilers. For long-term (>20 years) case including liquid hot water (LHW) pretreatment and consolidated bioprocessing (CBP, see Section 4.4), a total energy efficiency of 52% and ethanol production costs of 8.7 €/GJ are expected; the energy efficiency is lower compared with the mid-term case because of the increased conversion of the polysaccharides that decrease the amount of biomass that would be burned in the boilers for power generation. It is evident the role of process integration in the improvement of the overall process. These authors concluded that it is unlikely that ethanol be cost-competitive compared to fossil-derived gasoline (production costs of 4–6 €/GJ for oil prices in 2002) or methanol from biomass (5–7 €/GJ for long-term), although the experience of Brazil shows important implementation advantages. Polakovič and Mandenius (1995) have used the equation-oriented simulator SPEEDUP (Aspen Technologies, Inc., USA) for the analysis of ethanol production from dilute solutions of sugars. Based on the obtained results, these authors proposed the addition of a second more concentrated stream of sugars for the retrofitting of continuous plants utilizing such dilute solutions of sugars like spent liquor from paper and pulp mills.

Simulation confirms to be a powerful tool during the evaluation of process alternatives. Modeling and simulation imply large savings during experimentation in pilot plants taking into account that these tests would confirm the assumptions made in the steps of design, and not on the contrary, i.e., when the design of a given process is defined by the experimentation in predefined ranges (not always the best ones) of operation variables.

#### 2.4.2. Modeling of cellulose degradation

Cellulose conversion is an important issue to be analyzed during process design of ethanol production from lignocellulosic biomass, especially when cellulose hydrolysis and fermentation of formed glucose (SSF process) is the selected technological option. Undoubtedly, mathematical modeling of such integrated processes as SSF provides a fundamental insight for the simulation of different alternative technological configurations. The NREL has funded projects concerning the modeling of biomass-to-ethanol conversion as in the case of continuous SSF of wood performed by South et al. (1995). This study was based on the experimental data obtained in a previous work of these same authors using commercial fungal cellulases (South

et al., 1993). In this study, a kinetic model considering the cellulose conversion, the formation and disappearance of cellobiose and glucose, the formation of cells and the biosynthesis of ethanol, was structured. In addition, a Langmuir-type model taking into account the adsorption of cellulases on the solid particles of cellulose and lignin, and expressions describing the dependence of cellulose conversion on the residence time of non-soluble solid particles of biomass were considered. This last description confers great validity to the model since it provides a better approximation to real processes, which cannot be suitably explained by the traditional models for CSTR considering soluble substances.

Langmuir model is widely used for the description of adsorption processes involving cellulases due to the good adjustment to experimental data in most cases. In addition, it represents a simple mechanistic model that can be used to compare kinetic properties of various cellulase–cellulose systems. Kadam et al. (2004) utilized a Langmuir-type isotherm to describe the enzymatic hydrolysis of cellulose for the case of dilute-acid pretreated corn stover; in this model, the inhibition effect on cellulases of other sugars present in the biomass hydrolyzate as xylose was considered as well as the effect of temperature (through Arrhenius equation) and the dosage of β-glucosidase. Bernardez et al. (1993) studied the adsorption process of complexed cellulase systems (cellulosome) released by the anaerobic thermophilic bacterium *Clostridium thermocellum* onto crystalline cellulose, pretreated wood and lignin employing Langmuir description. Nevertheless, some experimental data indicate that the negative effect of lignin content in the hydrolyzate is not principally due to the enzyme partitioning between cellulose and lignin, suggesting that lignin hinders saccharification by physically limiting the enzyme accessibility of the cellulose (Meunier-Goddik and Penner, 1999). Hence, more structure-oriented modeling is needed as a mean to gain insight on biomass hydrolyzate's hydrolysis and its optimal operating conditions. Other models have been proposed since the union of the cellulases to the cellulose does not meet all the assumptions inherent to the Langmuir model. To this end, two-site adsorption models, Freundlich isotherms and combined Langmuir Freundlich isotherms have been proposed (Zhang and Lynd, 2004). Lynd et al. (2002) present in their wide review about the microbial cellulose utilization, a compilation of values of adsorption parameters for cellulases isolated from different microorganism and for diverse substrates. In that work, the kinetic constants for cellulose utilization by different microorganisms are reported as well. On the other hand, it has been shown that the intensity of the agitation in batch reactors has a little effect over cellulose hydrolysis when cellulose particles are suspended. Based on the analysis of the kinetic constants and on experimental data, it was concluded that the external mass transfer is not a limiting factor of the global process of hydrolysis. However, when the internal area is much greater than the external one, as in the case of most cellulosic substrates, it is probable that cellulases can remain entrapped in the pores provoking lower hydrolysis rates (Zhang and Lynd, 2004). These considerations are essential when mathematical representations of the process are being developed.

Zhang and Lynd (2004) reviewed in detail the works concerning the modeling of cellulose hydrolysis and point out that most of proposed models for the design of industrial systems fall in the category of semimechanistic models, i.e. models taking into account the substrate concentration or one of the enzymatic activities as a state variable. These models meet the requirement of including the minimum of necessary information for the description of the process. These authors emphasize that most kinetic models do not consider the changes in the hydrolysis rate during the course of the reaction, and that those models that do this, are based mainly on empirically adjusted parameters and not on a mechanistic approach. For instance, the model of SSF process developed for the case of unpretreated wastepaper using commercial cellulases and *S. cerevisiae* for both batch and two-stage continuous regimes (Philippidis and Hatzis, 1997) made use of an exponential decay term to describe the time-dependent decline in the rate of cellulose hydrolysis. With the help of an exhaustive sensitivity analysis, the model showed that further improvements in the fermentation stage do not have great influence on ethanol yield; in contrast, the digestibility of substrate (as a result of pretreatment), cellulase dosage, specific activity, and composition have a great effect on ethanol yield. This confirms that major research efforts should be oriented to the development of more effective pretreatment methods and production of cellulases with higher specific activity.

Half of the mechanistic models cited by Zhang and Lynd (2004) are based on Michaelis–Menten model, which is valid when the limiting substrate is in excess in relation to the enzyme. In addition, competitive inhibition is the most found in the literature mechanism, although a combination of both non-competitive and competitive mechanisms for different inhibition effects can be found (see for example Philippidis et al., 1993). Due to the importance of modeling, these authors highlight the need of developing functional models that include the adsorption, various state variables for substrate besides the concentration (for example, polymerization degree or amount of amorphous cellulose) and multiple enzymatic activities.

### *2.4.3. Fermentation dynamics during fuel ethanol production*

Process design methodologies should consider control and operation aspects of the processes to be implemented at industrial scale. Control and operation of fermentation processes have become a complex task due to the biological nature of the system, its multicomponent character, and the inherent oscillatory behavior of some regimes of cultivation. This task requires the development of dynamic models to estimate the behavior of the system in dependence on the

time. Considering that not all the commercial simulators can analyze batch processes, Pascal et al. (1992) point out that the formulation of dynamic models meeting this requisite is needed. In particular, these authors modeled the transformation of beet molasses into ethanol obtaining a panorama of process activity and degree of equipment occupation. Dynamic simulation is also required for the control of fermentation processes including those ones carried out in batch, fed-batch and continuous regimes. Through a non-structured mathematical model that considers four state variables (concentrations of cells, substrate, product, and $CO_2$ evolution rate), Thatipamala et al. (1996) developed an algorithm for the prediction of non-measurable state variables and critic parameters varying with time, which allowed the on-line estimation of these variables and the adaptive optimization of a continuous bioreactor for ethanol production.

Oscillatory behavior of fermentations imposes great challenges for bioprocess design. This behavior is characteristic of continuous cultures of *Z. mobilis* under certain conditions like specific dilution rates or ethanol concentrations in the broth. Several experimental runs with forced oscillations of *Z. mobilis* culture were carried out in order to formulate and test a model describing the oscillatory behavior (Daugulis et al., 1997; McLellan et al., 1999). The model makes use of the concept of "dynamic specific growth rate", which considers inhibitory culture conditions in the recent past affecting subsequent cell behavior. Through dynamic simulation, it was shown that the lag in the cells' response was the major factor contributing to the oscillations. Moreover, the change in morphology to a more filamentous form may explain the change in specific growth and product formation characteristics. However, Zhang and Henson (2001) point out that dynamic simulation has several limitations for analyzing the dynamic behavior of fermentation processes related to the fact that only a limited number of simulations tests can be performed and that it does not easily reveal the model characteristics leading to certain dynamic behaviors. In contrast, non-linear analysis allows a deeper insight of this type of processes. Non-linear analysis provides tools for studying the appearance of multiple steady states with changes in parameter values of the model. These authors performed the bifurcation analysis for models describing continuous alcoholic fermentation of *Z. mobilis* and *S. cerevisiae* and concluded that utilized tools allowed revealing important characteristic of the employed models as the lack of model robustness to small parameter variations and the coexistence of multiple stable solutions under the same operating conditions. An experimentally verified, unsegregated, two-compartment model of the ethanol fermentation was utilized to assess the dynamic behavior of a stirred-tank bioreactor with a membrane for the *in situ* removal of ethanol (Garhyan and Elnashaie, 2004; Mahecha-Botero et al., 2005, 2006). Through bifurcation analysis, it was shown that the operation of the reactor under periodic/chaotic attractors conditions gives higher substrate conversions, yields and production rates than the corresponding steady states. It also has been shown that the membrane acts as a stabilizer of the process eliminating the oscillations.

The operation of bioreactors in fed-batch mode is very difficult to model due to the fact that microbial cells grow under permanently changing conditions. To tackle this problem, da Silva et al. (1999a) developed a hybrid neural model for the alcoholic fermentation by *Z. mobilis* in fed-batch regime. The model uses all the available information about the process to deal with the difficulties in its development and could be the base for the formulation of the optimal feed policy of the reactor. Kapadi and Gudi (2004) developed a methodology for the determination of optimal feed rates of culture medium during fed-batch fermentation using differential evolution resulting in a predicted augment of ethanol concentration at the end of each cultivation cycle. Wang and Jing (1998) developed a fuzzy-decision-making procedure to find the optimal feed policy of a fed-batch alcoholic fermentation using recombinant yeasts able to assimilate glucose and xylose. The kinetic model involved expressions that take into account the loss of plasmids. For solving this problem, a Hybrid Differential Evolution (HDE) method was utilized. The application of HDE has been also carried out in order to simultaneously determine the optimal feed rate, fed glucose concentration and fermentation time for the case of *S. diastaticus* during ethanol production. The optimal trade-off solution was found using a fuzzy goal attainment method that allowed obtaining a good agreement between experimental and computed results (Chen and Wang, 2003). HDE has been also used for the estimation of kinetic parameters during batch culture for the mentioned yeast (Wang et al., 2001). Other strategies of optimization have demonstrated their usefulness for the evaluation of hybrid configurations involving reaction–separation integration. For instance, vacuum fermentation technology has been modeled, simulated and optimized by means of factorial design and response surface analysis (da Silva et al., 1999b).

It is clear that the application of all described tools for the design of high-performance technological configurations for fuel ethanol production is required. This complex issue entails the improvement and development of tools for describing the dynamic behavior of the fermentation systems (e.g., implementation of bifurcation analysis techniques) and for process optimization (e.g. MINLP tools, optimization-based process synthesis and optimization under uncertainty), among others. Certainly, the application of advanced design techniques for process synthesis will allow achieving the development of innovative and alternative technological configurations, which consider the possibilities that offers the intensification of unit processes and operations by means of different kinds of integration: reaction–reaction, reaction–separation, separation–separation, and energy integration (see Sections 4–7).

## 3. Cogeneration and co-products

Process engineering looks for the design of high-performance processes meeting several and, sometimes, contradictory criteria. During process design, the evaluation of alternative flowsheets should be aimed not only at reaching higher conversions of raw materials and productivity, but also at taking advantage of the by-products released during the transformation of feedstocks converting them into valuable co-products. In this way, economical and even environmental criteria may be met. Various streams, mostly of organic nature, are obtained during the processing of such materials as sugar cane, corn or lignocellulosic biomass. These organic materials have an important value either as a fuel or as source of other value-added products. To offset the inherent high cost of processing biological materials, the possibilities for producing co-products should be taken into account when designing ethanol production processes. In the present work, these possibilities are highlighted in the case of the three main types of feedstock studied.

### 3.1. Thermal conversion of biomass

Due to the relative high costs of ethanol from biomass and other feedstocks, different strategies are being developed for making the process more profitable. In the specific case of lignocellulosic biomass, the thermal conversion of non-fermentable lignin produced as a by-product can provide the energy required by the entire process remaining a surplus that can be commercialized in form of electricity. This is possible due to the high energy value of the lignin (29.54 MJ/kg) that is released during its combustion. Reith et al. (2001, 2002) point out that the use of BIG/CC technology for the thermal conversion of the non-fermentable residues can supply all the steam and electricity needed by the biomass-to-ethanol process. In addition, the electricity surplus can be sold to the grid giving a total system efficiency of 56–68%. In this point, the cogeneration of steam and electricity is crucial for obtaining a competitive process. In addition, if the global process is energetically integrated, the amount of generated electricity available for sale will be larger (Wyman, 1994). Other residues from lignocellulosic process can be used for steam or electricity generation. Eriksson et al. (2004) employed the solid residue obtained from the two-stage pretreatment of spruce sawdust with dilute sulfuric acid for the generation of electric energy. The solid residue was separated from the liquid by centrifugation and then dried and powdered. Obtained results showed that a gas turbine designed for wood could use this residue as a fuel.

Carrocci and James (1991) proposed and analyzed two cogeneration systems for improving the global efficiency of ethanol production plants in Brazil. Studied systems can be implemented using available and mature technology at relatively low prices. The analysis was done for a plant producing 210,000 L/d of ethanol. The first system comprises the utilization of 2,700,000 L/d of vinasses for generation of biogas, which can be converted in electricity through a conventional cycle of thermodynamic cogeneration. Part of fibrous residues (474,000 kg/d), mainly bagasse, is used for steam and electricity production for the plant, whereas the other part (276,000 kg/d) is converted in electricity for surrounding zones. In the second system, the biogas is utilized as a fuel for trucks, including those that transport the sugar cane to the plant. The energetic analysis demonstrated that the first option is slightly more efficient than the second, but for the Brazilian economy could be more important the supply of a fuel created in the same plant for the mobilization of heavy transport. According to data of Moreira (2000), 80 L of ethanol and 280 kg (50% moisture) of bagasse can be obtained from 1 ton of sugarcane. In addition, 6.7 kg of steam are required for obtaining one kW h (assuming 20% efficiency for the conversion of steam to electricity). If considering that 1 kg of steam requires the combustion of 0.45 kg of bagasse (Prakash et al., 1998), the amount of electricity that can be generated from 1 kg of bagasse is 0.33 kW h. Moreira (2000) emphasizes that Brazilian electricity market is opening the space for using large amounts of biomass residues obtained during ethanol production as a fuel in steam boilers. For example, if biomass gasifiers and gas turbines were installed in all sugar mills located in the state of São Paulo (Brazil), 6000 MW of electricity could be produced. Similarly, Gallagher et al. (2006) have proposed the generation of power from the biomass residues generated in dry-milling facilities for ethanol production. To this end, corn stover can be burned in order to generate electricity for the grid reducing ethanol production costs in dry mills if high natural gas prices are sustained during the next decade.

### 3.2. Current and potential co-products

New trends in process engineering of bioethanol are aimed at producing co-products other than fuels that contribute to balance the economy of the global ethanol production process. In this way, many materials generated during the process and considered as wastes could become valuable and marketable co-products. Logically, the type of co-products depends on the type of employed feedstock as shown in Table 1.

Ethanol production process using sugar cane allows the production of valuable co-products as bagasse, which has different applications as can be observed in Table 1. In addition, the recovery of added-value co-products as fructose or invertase has been proposed. This recovery implies the use of new equipments that makes necessary the execution of feasibility studies in order to assess the economic viability of these additional production lines. The utilization of the energy obtained from bagasse for the production of poly-3-hydroxybutiric acid through one integrated process producing biodegradable plastic, sugar and ethanol has been reported (Ohara, 2003).

Table 1
Current and potential added-value co-products that can be obtained during fuel ethanol production

| Co-product | Process | Stage where co-product is formed | Application | Remarks | References |
|---|---|---|---|---|---|
| Yeast | Ethanol from sugar cane | Centrifugation of culture broth after fermentation | Cattle feed supplement | | Maiorella et al. (1984) |
| Bagasse | Ethanol from sugar cane | Crushing of sugar cane | Feedstock for production of animal feed, enzymes, aminoacids, organic acids, pharmaceuticals, etc. | Solid-state fermentation | Pandey et al. (2000) |
| | | | Substrate for production of xylitol, single-cell protein, flavors, cellulases, ligninase and xylanases by submerged fermentation | Pretreatment is required | Aguilar et al. (2002), Pandey et al. (2000) |
| | | | Feedstock for production of activated carbon with exceptionally high adsorptive capacity | | Lutz et al. (1998) |
| Fructose | Ethanol from sugar cane | Selective fermentation coupled with pervaporation or membrane distillation | Sweetener for food industry | Mutant yeasts not able to assimilate fructose | Atiyeh and Duvnjak (2003), Di Luccio et al. (2002) |
| Invertase | Ethanol from sugar cane | Fermentation | Enzyme used for the production of inverted sugar in food industry; analytical tests | | Echegaray et al. (2000) |
| Corn fiber | Corn wet-milling process | Wet milling | Substrate for production of xylanases, arabinosidases, amylases, cellulases and proteases by *Aureobasidium* sp. | | Leathers (2003) |
| CCDS | Corn wet-milling process | Evaporation of thin stillage (liquid fraction obtained after centrifugation of stillage) | Food for non-ruminants (poultry and swine) | | Belyea et al. (1998) |
| | | | Fermentation medium for production of pullulan by *Aureobasidium* sp. or astaxanthin by *Phaffia rhodozyma* | US$20–25/kg pullulan | Bon et al. (1997), Leathers (2003), Wilkie et al. (2000) |
| Corn gluten meal | Corn wet-milling process | Wet milling | High value animal food, containing 60% protein, but no appreciable fat or oil | Commercialized co-product | Madson and Monceaux (1995) |
| Corn gluten feed | Corn wet-milling process | Wet milling | Animal feed concentrate of the residual fiber and CCDS containing about 20% protein and very little fat or oil | Commercialized co-product; low grade product compared to DDGS | Madson and Monceaux (1995) |
| DDGS | Corn dry milling process | Combination of evaporated thin stillage and solids obtained after centrifugation of stillage | High value feed for ruminants containing 27–35% protein | Commercialized co-product; US$120/ton DDGS | Belyea et al. (2004), Madson and Monceaux (1995) |
| Xylitol | Biomass-to-ethanol process | Xylose solutions obtained during pretreatment of lignocellulosic biomass can be converted into xylitol by chemical or biotechnological means; co-culture of *Saccharomyces cerevisiae* and *Candida tropicalis* | Anticariogenic sweetener, sugar substitute for diabetics | US$7/kg xylitol | Converti and Del Borghi (1996), Latif and Rajoka (2001), Leathers (2003), Saha (2003) |
| 2,3-butanediol | Biomass-to-ethanol process | Arabinose and xylose solutions obtained during pretreatment of lignocellulosic biomass (especially corn fiber) can be converted into 2,3-butanediol by bacteria | Chemical feedstock as a precursor of synthetic polymers and resins | | Saha (2003) |
| CMA | Biomass-to-ethanol process | Xylose solutions obtained during pretreatment of lignocellulosic biomass can be converted into acetic acid by fermentation using *Clostridium thermoaceticum* | Road deicer | Stillage could be used to supply nutrients for fermentation process | Bungay and Peterson (1992), Wilkie et al. (2000) |
| Furfural | Biomass-to-ethanol process | Xylose solutions obtained during pretreatment of lignocellulosic biomass can be converted into furfural | Valuable chemical | US$1580/ton furfural | Kaylen et al. (2000) |

Table 1 (*continued*)

| Co-product | Process | Stage where co-product is formed | Application | Remarks | References |
|---|---|---|---|---|---|
| Single cell protein | Biomass-to-ethanol process | Xylose solutions obtained during pretreatment of lignocellulosic biomass can be utilized for growing *Candida utilis* | Animal feed | | Ghosh and Ghose (2003) |
| Unaltered lignin | Biomass-to-ethanol process | Delignification of biomass by solvent pretreatment | Fuel additive | US$200/ton | Ghosh and Ghose (2003) |
| Pelletized hydrolysis residue | Biomass-to-ethanol process | Dilute acid pretreatment of wood | Fuel pellets for residential appliances (stoves, burners) | | Öhman et al. (2006) |
| Lignin | Biomass-to-ethanol process | Fractionation of pretreated biomass or centrifugation of stillage | Raw material for production of adsorptive materials by chemical modification | | Dizhbite et al. (1999) |

CCDS, corn condensed distiller's solubles; DDGS, dried distiller's grains with solubles; CMA, calcium magnesium acetate.

When corn is utilized for ethanol production by wet-milling process and before ethanol processing, the main components of the grain are recovered. In this way, only starch enters the process. In 2004, ethanol producers that employ this technology in USA produced 426,400 ton of corn gluten meal, 2.36 mill ton of corn gluten feed and germ meal, and 560 mill pounds of corn oil (Renewable Fuels Association, 2005). Corn fiber mainly contains 30-50% of arabinoxylan, 15–20% of cellulose, 10–25% of attached starch and 11–12% of protein. This fiber could be used for enzyme production by fermentation processes using microorganisms able to assimilate the arabinose (Leathers, 2003). The range of products that could be obtained from a modern biorefinery employing the wet-milling technology includes ethanol, high fructose corn syrup, corn oil, corn gluten meal, corn gluten feed, and different chemicals and food-related products as vitamins and aminoacids (Lynd et al., 1999). From 100 kg of corn processed by this technology, 2.87 kg of corn oil, 4.65 kg of gluten meal, and 24.09 kg of gluten feed are produced (Bothast and Schlicher, 2005).

When dry milling of corn is employed during fuel ethanol production, all components of the grain (starch, fiber, proteins, fats, minerals) are involved in the process. Thin stillage represents the liquid fraction obtained after centrifugation or pressing of whole stillage. The solid fraction contains remaining corn and fermentation solids. Thin stillage can be concentrated by evaporation in order to obtain syrup of concentrated solubles that are mixed with the remaining solids. For increasing the shelf-life, the resulting product is further dried to 10–12% moisture forming the so-called Dried Distiller's Grains with Solubles (DDGS) that are widely utilized for animal feed. During the dry-milling processing of corn, 30.36 kg of DDGS are generated from 100 kg of corn (Bothast and Schlicher, 2005). Over 85% of DDGS has been fed to dairy and beef cattle as a high quality, economical feed ingredient. With continuing research, DDGS use in swine and poultry diets is expanding also. Approximately, 20–25% of DDGS are commercialized locally in wet form, reducing energetic costs and offering an additional market for ethanol producers. In 2004, ethanol production plants using dry-milling process in the USA produced 7.3 mill ton of DDGS. USA exports about 1 mill ton of DDGS mainly to Ireland, United Kingdom, other European countries, Mexico and Canada (Renewable Fuels Association, 2005). However, one of the most important factors affecting the market value of DDGS is the variation in their composition; for example, protein content (the most costly component in animal rations) of DDGS can vary from 27% to 35%. This variation is not related to variations in the composition of corn grain and can be explained by variations in the process streams or in processing techniques (Belyea et al., 2004). Taylor et al. (2000b) reported a hybrid process between wet-milling and dry-milling processes in which a fraction of corn oil and germ is obtained together with DDGS. This process contributes to the reduction of the production costs of each liter of ethanol in 0.53–1.06 US cents. The fractionation of corn before the fermentation is one approach to reduce DDGS volume and improve quality. With lower fiber and higher protein contents, modified DDGS resulting of the utilization of this technology can be used as feed for non-ruminants like poultry and swine. However, the removal of corn lipids implies the reduction of subsequent fermentation performance. For this reason, some lipid supplements have been tested during SSF of corn starch (Murthy et al., 2006). On the other hand, the nutritional quality of Corn Condensed Distiller's Solubles (CCDS), the syrup of concentrated solubles when corn wet-milling process is employed for producing ethanol, has been evaluated in order to utilize them for animal feed (Belyea et al., 1998). CCDS contain about 18% of protein and 20% of carbohydrates, including starch oligosaccharides that did not undergo saccharification. CCDS also have growth factors as vitamins and peptides. For this reason, CCDS could be considered as a fermentation medium for the production of valuable substances (see Table 1). When molasses are used as a feedstock, the evaporation of stillage leads to the production of syrup called Condensed Molasses Solubles, which is a low-value feed

additive with a high content of potassium, limiting its use in feed formulations due to the laxative effect (Wilkie et al., 2000).

In the case of ethanol production from lignocellulosic biomass, it is considered that configurations allowing the recovery of different co-products could significantly improve the process economy, but to date, these technologies could not be competitive without the help of tax exemptions (Ancillotti and Fattore, 1998). Rubio et al. (1998) have suggested that the pentose-rich liquid streams obtained during the pretreatment of biomass, for example by steam explosion, can be utilized for the production of chemical products as xylitol and furfural. The IIT Delhi process involves the use of this xylose-rich stream to grow *Candida utilis* for its subsequent use as animal feed (Ghosh and Ghose, 2003). This model process considers the production of unaltered lignin with a high molecular weight thanks to the employ of solvent pretreatment of biomass. The lignin obtained in this way can be commercialized as a high value fuel additive instead of burning it for steam and/or power generation. The Lignol process also contemplates the production of high quality sulfur-free lignin (in comparison to lignosulfonates and thiolignins recovered from sulfite and Kraft processes in the pulp and paper industries). One of the Lignol's current research directions is oriented to the potential applications of this kind of lignin in the production of adhesives, concrete plasticizers, friction materials for brake products, grease, and asphalt antioxidants and coatings (Arato et al., 2005). Regarding the lignin obtained in the process from biomass, Wyman (1994) points out that the phenolic compounds originated during the lignin degradation could react with alcohols to form methyl or ethyl aryl ethers that could be utilized as octane boosters. These products would be more valuable than the use of lignin as a boiler fuel, but conversion technologies should be sufficiently low in costs to make them economically viable. On the other hand, lignin can be converted in a great variety of chemical products, but one of the limitations is the reduced market for these lignin co-products. Pan et al. (2005) suggest that the high content of phenolic hydroxyl groups present in the lignin extracted from the softwood biomass pretreated by the organosolv process indicates the potential of this pretreatment method for the production of phenolic, epoxy, and isocyanate resins.

Process synthesis can supply the necessary information in order to assess if alternative configurations involving the diversification of such products within a bioethanol production plant are technically, economically and environmentally viable. Kaylen et al. (2000) reviewed the most promising technologies for ethanol production from biomass and selected a scheme involving dilute-acid pretreatment, conversion of released pentoses into furfural, concentrated-acid hydrolysis of cellulose, hexose fermentation using yeasts and recovering $CO_2$, distillation, anaerobic treatment of effluents collecting the generated methane, and recovery of remaining cellulose and lignin. Based on pilot-plant data, these authors carried out a feasibility study considering the use of energy crops, crop residues and wood biomass as feedstocks. The obtained results indicate that the production of ethanol as a single product is not viable, while the co-production of ethanol and furfural appears to be very profitable. The utilized model maximized the net present value of the plant and allowed the definition of the optimal plant size and location. Crop residues were the most perspective feedstock. This represents a step towards the development of a biorefinery that could involve the production of several combined products. Wyman (2003) also presents a techno-economical analysis of such cellulosic biorefinery that can have great benefits derived from the economy of scale producing a large market product as the fuel ethanol, other co-products that can utilize the inexpensive sugars obtained from a large operation, and generating power for selling to the grid. This large facility could make use of the synergy provided by the combined production of fuels, chemicals and power at lower costs than if just one of these products were made. These works demonstrated the importance of process synthesis combined with optimization techniques and techno-economic evaluations in order to obtain valuable information for making decisions about the production of biofuels.

## 4. Reaction–reaction integration

Process design has been boosted due to process integration. In particular, when several operations can be carried out in a same single unit, the possibilities for improving the performance of the global process are higher. Rivera and Cardona (2004) classified the integrated processes into simultaneous, when they are carried out in a same equipment, or conjugated, when they are carried out in different equipments closing the technological scheme among these equipments through fluxes or refluxes. In addition, these authors classify the processes into homogenous, when two or more unit operations or two or more chemical reactions are combined, and heterogeneous, when one unit operation and one or more chemical reactions are combined simultaneously. Simultaneous processes allow a mutual intensification and an augment of their possibilities as well as the development of more compact technological schemes. In this context, extractive distillation is an example of a simultaneous homogenous process, whereas the coupling of the fermentation with the pervaporation is a conjugated heterogeneous process. All these designs allow the integration among the steps of an overall process. For instance, the combination in a same unit of the enzymatic hydrolysis and the microbial transformation leads to the reduction of the negative effect due to the inhibition of the enzymes by the product of the reaction catalyzed by them; this correspond to an integration of the reaction–reaction type.

There exist different possibilities for reaction–reaction integration during production of ethanol from lignocellulosic

biomass (Fig. 1) and from starch (Fig. 3). Some examples illustrating the reaction–reaction integration processes applied to the production of fuel ethanol from different feedstocks are shown in Table 2. In general, reaction–reaction integration has been proposed for the integration of different biological transformations taking place during ethanol production. Nevertheless, this type of integration can be done between different steps of ethanol production process involving chemical transformations as well. In this sense, the integration of detoxification step with fermentation can play an important role in their intensification and in the reduction of ethanol production costs. For instance, Palmqvist and Hahn-Hägerdal (2000) propose a way to implement this kind of integration consisting in carrying out the detoxification in the same vessel in which fermentation is to be accomplished just before the cultivation process.

### 4.1. Co-fermentation of lignocellulosic hydrolyzates

Considering as the starting point the non-integrated separate hydrolysis and fermentation process, several types of reaction–reaction integration can be analyzed. Among the first efforts of integration, the co-fermentation using mixed cultures of different compatible species can be mentioned. This kind of integration is oriented to the complete assimilation by the microorganisms of all the sugars previously released during the pretreatment and hydrolysis of lignocellulosic biomass (see Fig. 1). The use of mixed cultures of yeasts that assimilate both hexoses and pentoses has been proposed, but problems related to the fact that hexose-utilizing microorganisms grow faster than pentose-utilizing microorganisms and that the conversion of hexoses to ethanol is consequently more elevated, are arisen. One approach to solve this type of problems is the employ of respiratory deficient mutants of the hexose-fermenting microorganisms with the aim of elevating the fermentation and growth activities of the pentose-fermenting microorganisms, which are very low when they are cultivated along with rapid hexose-fermenting yeasts. In addition, the presence of hexose-fermenting microorganisms allows the reduction of the catabolic repression on the pentose consumption by the pentose-assimilating microorganisms (Laplace et al., 1993). Considering the indicators for the process using only the glucose-assimilating bacterium *Z. mobilis* grown on the biomass hydrolyzate, the productivities of the mixed culture are less than those of the bacterium, but the yields are comparable, which offers a space for further research (Delgenes et al., 1996). One of the problems that lies in this kind of configurations is that pentose-fermenting yeasts present a greater inhibition by ethanol that limits the use of concentrated substrates.

Other variant of co-fermentation consists in the utilization of a single microorganism capable of assimilating both hexoses and pentoses in an optimal way allowing high conversion and ethanol yield. Although in the nature these microorganisms exist, a high efficiency in the conversion to ethanol can be reached through the genetic modification of yeasts or bacteria already adapted to the ethanolic fermentation. The microorganisms most commonly modified

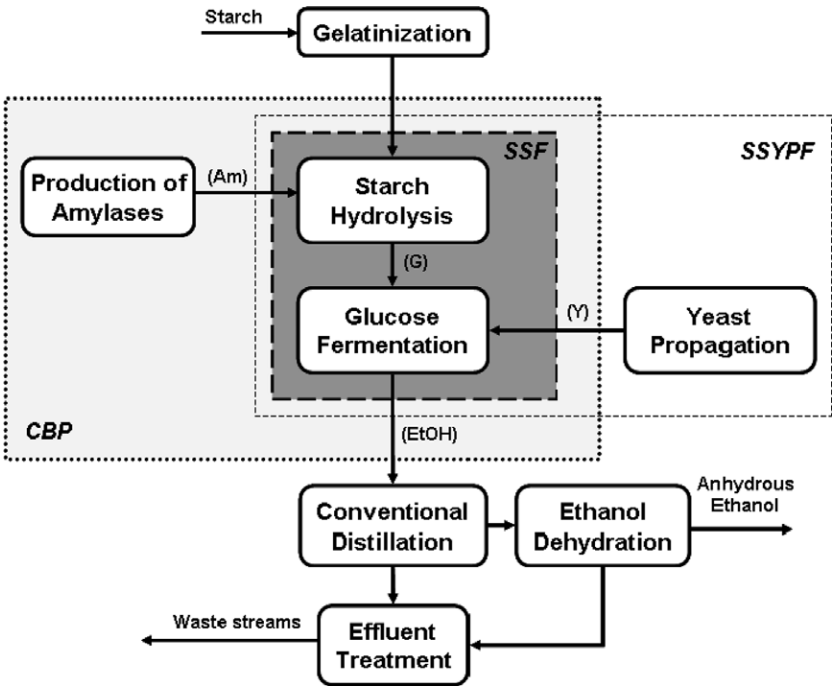


Fig. 3. Generic block diagram of fuel ethanol production from starchy materials. Possibilities for reaction–reaction integration are shown inside the shaded boxes: SSF, simultaneous saccharification and fermentation; SSYPF, simultaneous saccharification, yeast propagation and fermentation; CBP, consolidated bioprocessing. Main stream components: Am, amylases; G, glucose; Y, yeasts; EtOH, ethanol.

Table 2
Process intensification through reaction–reaction integration for bioethanol production

| Type of intensification process[a] | Technology | Bioagent/unit process | Feedstock/medium | Remarks | References |
|---|---|---|---|---|---|
| Simultaneous | Co-fermentation (mixed culture) | *Saccharomyces cerevisiae* mutant + *Pichia stipitis* | Glucose and xylose | Batch and continuous cultures; 100% glucose conversion and 69% xylose conversion | Laplace et al. (1993) |
| | | Respiratory deficient *S. diastaticus* + *P. stipitis* | Steam-exploded and enzymatically hydrolyzed aspen wood | Continuous culture; EtOH conc. 13.5 g/L, yield 0.25 g/g, productivity 1.6 g/(L h); 100% conversion of glucose and xylose | Delgenes et al. (1996) |
| Simultaneous | Batch detoxification and enzyme production | *Trichoderma reesei* | Steam-pretreated hemicellulose hydrolyzate of willow | Fungus utilizes the hydrolyzate's pentoses as substrate and removes inhibitors simultaneously | Palmqvist et al. (1997) |
| Simultaneous | Batch hydrolysis and solid substrate fermentation (mixed culture) | Thermotolerant *S. cerevisiae* + *Bacillus* sp. | Sweet sorghum, sweet potatoes | Bacterium produces amylases for starch hydrolysis; formed sugars are converted into ethanol by yeast; EtOH conc. 5 g/100 g substrate; 37 °C; 72 h cultivation | Sree et al., 1999 |
| Simultaneous | Xylose isomerization and fermentation | *S. cerevisiae* + xylose(glucose)-isomerase | Non-pretreated spent sulfite liquors, acid-hydolyzed wheat straw | Yield 0.41 g/g; 51–84% xylose utilization; batch process | Chandrakant and Bisaria (1998), Lindén and Hahn-Hägerdal (1989) |
| Simultaneous | Batch SSF (mixed culture) | *S. cerevisiae* + *Fusarium oxysporum* | Sweet sorghum stalks | Fungus produces cellulases and hemicellulases for hydrolysis process; formed sugars are converted into ethanol by concerted action of both microorganisms; 108–132% yield; EtOH conc. 35–49 g/L. | Mamma et al. (1995), (1996) |
| Simultaneous | Batch SSF (mixed culture with co-product formation) | *S. cerevisiae* + *Candida tropicalis* + *Chaetomium thermophile* cellulases and xylanases | Alkali pretreated corn cobs | *C. tropicalis* produces xylitol and ethanol; EtOH conc. 21 g/L, xylitol conc. 20 g/L; EtOH yield 0.32 g/g, xylitol yield 0.69 g/g; 37 °C | Latif and Rajoka (2001) |
| Simultaneous | Batch SSF | *S. cereveisiae* + *Aspergillus niger* glucoamylase | Raw wheat flour | Previous liquefaction with α-amylase; 21–31 h cultivation; EtOH conc. 67 g/L | Montesinos and Navarro (2000) |
| | | Yeasts + *T. reesei* cellulases supplemented with β-glucosidase | Pretreated lignocellulosic biomass | 3–7 d of cultivation; EtOH conc. 40–50 g/L for *S. cerevisiae*, 16–19 g/L for *Kluyveromyces marxianus*; 90–96% substrate conversion | Ballesteros et al. (2004), De Bari et al. (2002), Hari Krishna et al. (1998), Lynd et al. (2001), South et al. (1993), Wyman (1994) |
| Simultaneous | Semicontinuous SSF | *S. cerevisiae* + commercial cellulase supplemented with β-glucosidase | Paper sludge | Special design of solids-fed reactor; EtOH conc. 35–50 g/L; 0.466 g/g EtOH yield; 74–92% cellulose conversion; 1–4 months of operation | Fan et al. (2003) |

| | | | | | |
|---|---|---|---|---|---|
| Simultaneous | Continuous SSF | *S. cerevisiae* + microbial amylases | Grains | Yield 2.75 gal/bushel; industrially implemented | Madson and Monceaux (1995) |
| | | Co-immob. *Zymomonas mobilis* and glucoamylase on κ-carrageenan | Liquefied corn liquid | Fludized-bed reactor; approx. 100 g/L dextrin feed; conversion 53.6–89.3%; EtOH conc. 22.9–36.44 g/L; productivity 9.1–15.1 g/(L h) | Krishnan et al. (1999a) |
| | | *S. cerevisiae* + commercial cellulase supplemented with β-glucosidase | Dilute-acid pretreated hardwood | CSTR; residence time 2–3 d; 83% conversion; EtOH conc. 20.6 g/L | South et al. (1993) |
| Simultaneous | Continuous SSYPF | *S. cereveisiae* + microbial amylases | Corn, milo, wheat | Yield 2.75–2.8 gal/bushel; industrially implemented | Madson and Monceaux (1995) |
| Simultaneous | Batch SSCF | Recombinant *Z. mobilis* + *T. reesei* cellulases | Dilute-acid pretreated yellow popplar | EtOH produced 17.6–32.2 g/L; yield 0.39 g/g; productivity 0.11–0.19 g/(L h) | McMillan et al. (1999) |
| Simultaneous | Continuous SSCF | Recombinant *Z. mobilis* + *T. reesei* cellulases | Dilute-acid pretreated wood chips | Cascade of reactors; model process of the NREL; 92% glucose conversion, 85% xylose conversion | Wooley et al. (1999a) |
| Simultaneous | Batch CBP | *Clostridium thermocellum* + *C. thermosaccharolyticum* | Lignocellulosic biomass | First bacterium produces cellulases and converts formed glucose into ethanol; second bacterium converts pentoses into ethanol; by-products formation; EtOH conc. 30 g/L; low ethanol tolerance | Claassen et al. (1999), Lynd et al. (2002), McMillan (1997), Wyman (1994) |
| | | *F. oxysporum* | Cellulose | Anaerobic conditions; yield 0.35g/g cellulose, productivity 0.044 g/(L h) | Panagiotou et al. (2005a) |
| Simultaneous | Continuous CBP | Recombinant *S. cerevisiae* | Starch-containing medium | Immobilized cells in calcium alginate; yeast expresses glucoamylase and converts starch into ethanol; EtOH conc. 7.2 g/L; 200 h cultivation | Kobayashi and Nakamura (2004) |
| Conjugated | Bioethanol and biomethanol production | *S. cerevisiae*/catalytic hydrogenation of $CO_2$ | Sweet sorghum | $CO_2$ released during ethanolic fermentation is used for methanol production; conceptual proposal for large ethanol production facilities | Grassi, 1999 |
| Conjugated | Semicontinuos two-stage hydrolysis-fermentation system | Hydrolysis with *A. niger*/ fermentation with *S. cerevisiae* | Potatoes starch | Two bioreactor connected through biofilter for removal of fungal biomass; 206 h cultivation; 3 loads of feeding; EtOH conc. 13.1–34.2 | León et al. (1997) |

CBP, consolidated bioprocessing; EtOH conc., ethanol concentration at the end of batch culture or in the effluent for continuous processes; SSCF, simultaneous saccharification and co-fermentation; SSF, simultaneous saccharification and fermentation; SSYPF, simultaneous saccharification, yeast propagation and fermentation.

[a] According to the classification of Rivera and Cardona (2004).

for this purpose are *S. cerevisiae* and *Z. mobilis* to which genes allowing the assimilation of pentoses have been introduced. The other approach for genetic modification is the introduction of genes encoding the metabolic pathways for the production of ethanol to microorganisms that are capable of fermenting both hexoses and pentoses in their native form. The "design" of ethanologenic bacteria like *E. coli* or *K. oxytoca* are examples of such type of modification. The employ of these recombinant microorganisms allows implementing co-fermentation processes intended to the more complete utilization of the sugars contained in the hydrolyzates of lignocellulosic biomass. The review of the different aspects related to the construction of genetically modified strains for bioethanol production is beyond the objectives of this work. However, the reader can consult the illustrative works of Aristodou and Penttilä (2000), Lynd et al. (2002), and Zaldivar et al. (2001).

Besides the understanding of the aspects related to the molecular biology of these microorganisms and from the viewpoint of process integration, it is necessary a deeper study of the relationships of this co-fermentation process through modeling and simulation in order to define the optimal cultivation conditions for increasing productivity and conversion of biomass into ethanol. Thus, the significant amounts of glucose and xylose present in the lignocellulosic hydrolyzates should be completely fermented in order to make the process economically viable. Olsson et al. (1995) proposed a simple kinetic model for describing ethanol formation from either glucose or xylose, or from a mixture of these two sugars utilizing as fermenting organism a recombinant ethanologenic strain of *E. coli* in batch regime. The model considers the mutual inhibition of sugar utilization rates and corresponding ethanol production rates. The global effect of inhibitors was also taken into account, which allows the evaluation of the effect of detoxification step on fermentation. However, there exists no kinetic dependence for cell growth rate in the proposed model; this makes difficult its application to different technological configurations for ethanol production design. Leksawasdi et al. (2001) developed a useful non-structured model describing the consumption of glucose and xylose when these sugars are present simultaneously in the culture medium as well as the cell growth and ethanol formation during batch fermentation using a recombinant *Z. mobilis* strain. The model takes into account the limitation of the process by high substrate concentrations and the inhibition by substrate and product. Similarly, Krishnan et al. (1999b) formulated a model for the fermentation of sugars mixture of glucose and xylose incorporating the effects of substrate inhibition, product inhibition, and inoculum size from cultivation data using a recombinant *Saccharomyces* strain. Hodge and Karim (2002) developed and validated a model for ethanol production from glucose and xylose using recombinant *Z. mobilis*. The model considered different inhibitions functions fitted by polynomial functions accounting the effect of ethanol, pH and both substrates on the kinetic rates. The model was applied to control the product concentration during fed-batch fermentation in order to offset the inhibitory effects. The optimal feed policy obtained on line allowed 16.6% improvement in ethanol productivity in comparison to the batch operation.

### 4.2. Simultaneous saccharification and fermentation

One of the most important advances related to the overall process of ethanol production from different feedstocks, is the implementation and development of the simultaneous saccharification and fermentation process (SSF), in which the enzymatic degradation of cellulose (see Fig. 1) or starch (see Fig. 3) is combined with the fermentation of glucose obtained from the hydrolysis of these polysaccharides. The key of the SSF from biomass is its ability for rapidly converting the sugars into ethanol as soon as they are formed diminishing their accumulation in the medium. Bearing in mind that the sugars are much more inhibitory for conversion process than ethanol is, SSF can reach higher rates, yields and ethanol concentrations in comparison with SHF (Wyman et al., 1992). SSF offers an easier operation and a lower equipment requirement than the sequential process since no hydrolysis reactors are needed; moreover, the presence of ethanol in the broth makes that the reactioning mixture be less vulnerable to the action of undesired microorganisms (Wyman, 1994). Nevertheless, SSF has the inconvenient that the optimal conditions for hydrolysis and fermentation are different, which implies a difficult control and optimization of process parameters (Claassen et al., 1999); in addition, larger amounts of exogenous enzymes are required.

The concept of SSF process was firstly described by Takagi et al. (1977). Takagi, Suzuki and Gauss had previously patented the SSF technology for bioethanol production (Gauss et al., 1976) by which the yeasts metabolize simultaneously the glucose into ethanol *in situ* during the enzymatic saccharification of the cellulose. This patent expired in 1993 and it has been utilized for small-scale demonstrations according to Ingram and Doran (1995), but until now, no commercial plants have been built at industrial level. Recently, Abengoa Bioenergy announced the start of the operation of the world's first commercial demonstration facility for ethanol production from biomass by the end of 2006; wheat straw will be the utilized feedstock and the plant will have a capacity of 5 mill liters per year, but the conversion technology will involve the employ of the SHF process (Abengoa Bioenergy, 2005). Since the time in which the first experiences with SSF from biomass began, the duration of the batch process have decreased from 14 d required for the conversion of 70% of cellulose into ethanol with final concentrations of 20 g/L, to 3–7 d needed for reaching 90–95% conversions with final ethanol concentrations of 40–50 g/L (Wyman, 1994).

Due to the elevated cost of the commercial cellulases, the integration of the cellulase production process using *T. reesei* with ethanolic fermentation has been proposed. As a small amount of enzymes remains entrapped in fungal cells producing cellulases, the addition of the whole culture broth of this process to the SSF reactor was proposed. Besides the fungal biomass and obtained cellulases, this broth contains residual cellulose and lignin. This allows the increase of the β-glucosidase activity, essential for the reduction of cellobiose levels, and a more complete utilization of sugars employed during the production of cellulases (Wyman, 1994).

As noted above, one of the major drawbacks of the SSF from biomass consists in the different optimal conditions, mainly pH and temperature, for the hydrolysis of cellulose and fermentation. Cellulases work in an optimal way at 40–50 °C and pH of 4–5 whereas the fermentation of hexoses with *S. cerevisiae* is carried out at 30 °C and pH of 4–5, and fermentation of pentoses is optimally performed at 30–70 °C and pH of 5–7 (Olsson and Hahn-Hägerdal, 1996). One solution to this disjunctive is the utilization of thermotolerant yeasts like *Kluyveromyces marxianus* that can be cultured at 42–43 °C in a batch SSF process (Ballesteros et al., 2004; Hari Krishna et al., 2001). If, in addition, the yeasts can also assimilate pentoses, the SSF process can become more perspective. Yeasts as *Candida acidothermophilum*, *C. brassicae*, *S. uvarum* and *Hansenula polymorpha* can be used for these purposes. In this case, the addition of a larger amount of nutrients to the medium is required; alternatively, the utilization of higher cell concentrations could be implemented for obtaining better results (Olsson and Hahn-Hägerdal, 1996; Ryabova et al., 2003). The difficulty lies in the fact that higher temperatures enhance the inhibitory effect of ethanol. Therefore, the isolation and selection of microorganisms that could be adapted in a better way to these hard conditions should be continued. Kádár et al. (2004) make reference to several reports about the utilization of thermotolerant microorganisms for the ethanol production.

Other proposed approach is the utilization of mixed cultures in such a way that the hydrolysis and fermentation of lignocellulosic biomass be carried out simultaneously. This procedure was applied to sweet sorghum stalks employing cellulase- and hemicellulase-producing fungus *Fusarium oxysporum* along with *S. cerevisiae* (Mamma et al., 1995). Considering that sweet sorghum stalks contain several carbohydrates as sucrose, glucose, hemicellulose and cellulose, the obtained yields in this process were higher than the theoretical yield from only glucose (0.51 g EtOH/g glucose), that is explained by the additional bioconversion of cellulose and hemicellulose into ethanol (Mamma et al., 1996). However, the final ethanol concentrations in this type of SSF process were quite low considering the separation process. Logically, the mixed culture presents a high complexity during its implementation at industrial level and has the additional disadvantage that optimal growth conditions for two or more different microorganisms are not the same. Besides, part of the substrate is deviated for the growth of the enzyme-synthesizing microorganism. Panagiotou et al. (2005a) carried out the SSF of cellulose with *F. oxysporum* demonstrating the production of ethanol under anaerobic conditions. In addition, the metabolite profiling of the microorganisms cultivated in different media was achieved through the measurement of the intracellular concentration of key metabolites (Panagiotou et al., 2005a,b,c).

### 4.3. Simultaneous saccharification and co-fermentation

Other promising integration alternative is the inclusion of the pentose fermentation in the SSF, process called simultaneous saccharification and co-fermentation (SSCF) as depicted in Fig. 1. In an initial stage, the co-fermentation of mixed cultures was studied. For example, the co-culture of *P. stipitis* and *Brettanomyces clausennii* has been utilized for the SSCF of aspen at 38 °C and pH of 4.8 yielding 369 L EtOH per ton of aspen during 48 h batch process, as reported by Olsson and Hahn-Hägerdal (1996). In this configuration, it is necessary that both fermenting microorganisms be compatible in terms of operating pH and temperature. Chandrakant and Bisaria (1998) suggest that a combination of *C. shehatae* and *S. cerevisiae* is suitable for this kind of process.

Similarly, a system including the isomerization of xylose and the fermentation with *S. cerevisiae* in a simultaneous way can be utilized. In this case, the enzyme glucose-isomerase (that is a natural xylose–isomerase) converts the xylose into xylulose, which can be assimilated by the yeasts implying the co-fermentation of lignocellulosic biomass. This system has been proven in non-pretreated spent sulfite liquor and in pretreated acid-hydrolyzed wheat straw (Lindén and Hahn-Hägerdal, 1989) (see Table 2). This alternative has been indicated by Wyman (1994) and by Olsson and Hahn-Hägerdal (1996). Among the drawbacks of this configuration are the high by-product formation in the form of $CO_2$ and xylitol, poor enzyme stability, incompatible pH and temperature optima (pH of 7.0 and 70 °C for the isomerization process), and the reversibility of the enzyme transformation (Chandrakant and Bisaria, 1998).

Actual SSCF process has been demonstrated in the case of ethanol production from yellow poplar through a bench-scale integrated process that included the dilute-acid pretreatment of feedstock, conditioning of hydrolyzate for fermentation, and a batch SSCF (McMillan et al., 1999). In this case, the recombinant *Z. mobilis* assimilating xylose was used. SSCF is the process on which is based the technology designed as a model process by the NREL for the production of fuel ethanol from aspen wood chips (Wooley et al., 1999a). In this design, the utilization of recombinant *Z. mobilis* exhibiting a glucose conversion to ethanol of 92% and a xylose conversion to ethanol of 85% is proposed. Similarly, the employ of cellulases produced by *T. reesei* in the same plant at a rate of 15 FPU (filter paper units)/g cellulose is assumed. It is projected that SSCF can

be carried out in continuous regime with a residence time for the whole system of cascade fermentors of 7 d at 30 °C.

As in the case of SSF, the development of microbial strains able to growth at elevated temperatures may improve techno-economical indicators of SSCF process significantly. Wooley et al. (1999b) point out that the development of ethanologenic microorganisms capable to ferment at temperatures greater than 50 °C can potentially reduce the cost of cellulase enzyme by one-half considering that an increment of 20 °C during the saccharification may imply a doubling of the cellulose hydrolysis rate.

### 4.4. Consolidated bioprocessing

The logic culmination of reaction–reaction integration for the transformation of biomass into ethanol is the consolidated bioprocessing (CBP), known also as direct microbial conversion (DMC). The key difference between CBP and the other strategies of biomass processing is that only one microbial community is employed both for the production of cellulases and fermentation, i.e., cellulase production, cellulose hydrolysis, and fermentation are carried out in a single step (see Fig. 1). Lynd (1996) indicates that this difference has important consequences as the fact that no capital or operation expenditures are required for enzyme production within the process. Similarly, part of the substrate is not deviated for the production of cellulases. Moreover, the enzymatic and fermentation systems are entirely compatible. The improvement in the conversion technology is by far the item that most contributes to the costs reduction for ethanol production. According to projections by Lynd (1996), the reduction of production costs due to an advanced configuration involving the CBP is three times greater than the reduction related to the scale economy of the process and ten times greater than the reduction associated with a lower cost of the feedstock. This diminish would be accomplished thanks to the reduction of more than eight times in the costs of biological conversion (Lynd et al., 1996). Lynd et al. (2005) reported the comparative simulation of SSCF and CBP processes assuming aggressive performance parameters intended to be representative of mature technology. Their results indicate that production costs of ethanol for SSCF reach 4.99 US cents/L including the costs of dedicated cellulase production, whereas CBP gives total costs of only 1.11 US cents/L demonstrating the effectiveness of this process configuration.

Lynd et al. (1999, 2002) point out that most research on cellulose hydrolysis is being carried out within the context of the enzymatically oriented intellectual paradigm, which focuses on cellulose hydrolysis as primarily an enzymatic rather than microbial phenomenon. Therefore, this paradigm justifies the search of microorganisms that actively secrete cellulases. In contrast, the microbial oriented paradigm considers cellulose hydrolysis as a microbial phenomenon and anticipates the development of the CBP. In fact, the concept of CBP involves four biologically mediated transformations: the production of saccharolytic enzymes (cellulases and hemicellulases); the hydrolysis of carbohydrate components present in pretreated biomass to form sugars; the fermentation of hexose sugars (glucose, mannose and galactose); and the fermentation of pentose sugars (xylose and arabinose). These four transformations occur in a single step. In this case, a dedicated process for production of cellulases is not required that makes CBP a highly integrated configuration (Lynd et al., 2005). This process is conceptually depicted in Fig. 4 in the case of bioethanol production.

Wyman (1994) indicates that in most of the studies on CBP, the bacterium *C. thermocellum* is used for enzyme production, cellulose hydrolysis, and glucose fermentation, whereas the co-fermentation using *C. thermosaccharolyticum* allows the simultaneous conversion of pentoses obtained from hemicellulose hydrolysis into ethanol. In particular, the CBP using *C. thermocellum* showed a substrate conversion 31% higher than a system using *T. reesei* and *S. cerevisiae*. South et al. (1993) tested the DMC of cellulose into ethanol utilizing *C. thermocellum* showing, under very specific conditions with a residence time of 0.5 d, higher conversions than continuous SSF process. Some filamentous fungi as *Monilia* sp., *Neurospora crassa* and *Paecilomyces* sp. are also able to transform cellulose into ethanol (Szczodrak and Fiedurek, 1996). Nevertheless, this technique faces the problem of the low tolerance of clostridia to ethanol and the reduction in the ethanol yield due to the formation of acetic acid and salts of other organic acids like lactates (Baskaran et al., 1995; McMillan, 1997; Wyman, 1994). This makes that the final ethanol concentration be low in comparison with the traditionally used yeasts (0.8–60 g/L) with very large cultivation times of 3–12 days (Szczodrak and Fiedurek, 1996).

To date, there is no microorganism known that can exhibit the whole combination of features required for the development of a CBP as the one shown in Fig. 4. However, there are realistic expectations about the possibility of overcoming the limitations of current CBP organisms. Lynd et al. (2002) point out that the feasibility of a CBP process will be established when a microorganism or microbial consortium can be developed according to one of two strategies. The first of them, called native cellulolytic strategy (labeled "A" in Fig. 4), is oriented to engineer microorganisms having a high native cellulolytic activity in order to improve the ethanol production through the increase of their yield or tolerance, i.e., by the improvement of the fermentative properties of a good producer of cellulases. In this case, the modifications in the process microorganism should be aimed at reducing or eliminating the production of by-products as acetic acid or lactate, and at increasing the ethanol tolerance and titres. Recent studies have demonstrated the possibility to obtain ethanol-tolerant strains of *C. thermocellum* growing at ethanol concentrations exceeding 60 g/L, a titer sufficient not to

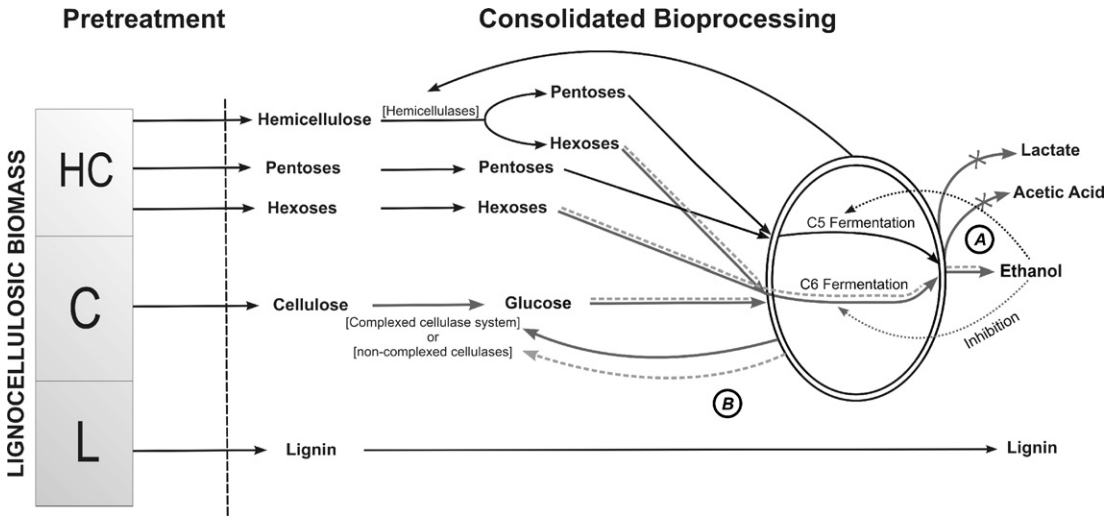


Fig. 4. Conceptual scheme of consolidated bioprocessing. (a) Native cellulolytic strategy. (b) Recombinant cellulolytic strategy. HC, hemicellulose; C, cellulose; L, lignin. Processing pathway for a thermoanaerobic microorganism (e.g. *Clostridium thermocellum*) is indicated by continuous gray lines. Processing pathway for an ethanologenic microorganism (e.g. *Zymomonas mobilis*) is indicated by dotted lines.

put thermophiles at a disadvantage relative to more conventional ethanol producers (Lynd et al., 2005).

The recombinant cellulolytic strategy (labeled "B" in Fig. 4) contemplates the genetic modification of microorganisms that present high ethanol yields and tolerances in such a way that they be capable of utilizing cellulose within a CBP configuration, i.e., making a microorganism with good fermentative properties to produce cellulases. It is considered that the second strategy has demonstrated to be more difficult due to the complexity of cellulases system (Béguin and Aubert, 1994). Currently, the production of cellulases by bacterial hosts producing ethanol at high yield as engineered *E. coli*, *K. oxytoca* and *Z. mobilis* and by the yeast *S. cerevisiae* has been studied. For instance, the expression of cellulases in *K. oxytoca* has allowed an increased hydrolysis yield for microcrystalline cellulose and an anaerobic growth on amorphous cellulose. Similarly, several cellobiohydrolases have been functionally expressed in *S. cerevisiae* (Lynd et al., 2005). Undoubtedly, ongoing research on genetic and metabolic engineering will make possible the development of effective and stable strains of microorganisms for converting cellulosic biomass into ethanol. This fact will surely imply a qualitative improvement in the industrial production of fuel ethanol in the future.

### *4.5. Reaction–reaction integration for ethanol production from starch*

In the case of fuel ethanol production from starchy materials, the reaction–reaction integration has also played an important role. SSF technology born in the 70s of the past century was assimilated by starch-processing industry for ethanol production obtaining high and sustainable yields of the order of 0.410 L/kg of corn (Madson and Monceaux, 1995). Fuel ethanol industry has advanced in this technology by incorporating the yeast propagation (from active dry yeasts) in the fermentor during initial saccharification, process named simultaneous saccharification, yeast propagation and fermentation (SSYPF) as depicted in Fig. 3. As in the process from biomass, high sugar concentrations are not achieved in the fermentor avoiding the inhibition of the enzymatic hydrolysis that is characteristic for the amylases as well. Due to this, the bacterial growth is inhibited because of the lack of substrate caused by the immediate conversion of glucose into ethanol. Maintaining the pH, nutrients and sterility in relation to bacteria, the complete conversion of available starch into ethanol is accomplished. SSYPF technology has been utilized in several plants in North America mainly employing corn, milo (a variety of sorghum), and wheat (Madson and Monceaux, 1995). Montesinos and Navarro (2000) have studied the possibility of utilizing raw wheat flour during batch SSF process with the aim of reducing costs attaining a decrease in the process time.

In the starch-to-ethanol industry, process steps related to the breakdrown of grain starch into glucose accounts 10–20% of the energy content of the fuel ethanol. One way to minimize this large amount of energy can be made by replacing high-temperature, liquid-phase, enzymatic hydrolysis with low-temperature, solid-phase, enzymatic hydrolysis. Robertson et al. (2006) have reviewed the so-called "cold hydrolysis" of the starch and concluded that the feasibility of this technology is linked to the discovery and characterization of more suitable enzymes and to the development of a process with higher level of integration such as the simultaneous liquefaction, saccharification and fermentation, among other factors.

Through recombinant DNA technology (genetic engineering), amylolytic yeast strains have been "constructed". This allows the design of ethanol production processes excluding the liquefaction and saccharification steps using

Table 3
Process intensification through reaction–separation integration for bioethanol production

| Type of intensification process[a] | Technology | Bioagent/unit operation features | Feedstock/medium | Remarks | References |
|---|---|---|---|---|---|
| Conjugated | Continuous vacuum fermentation | *Saccharomyces cerevisiae*/vacuum system | Glucose-containing medium | 50 mm Hg; with and without cell recycling; sparging of oxygen; 33.4% glucose feed; productivity 40–82 g/(L h) | Cysewski and Wilke (1977) |
| Conjugated | Continuous fermentation coupled with vacuum flashing | *S. cerevisiae*/extractive vacuum flash chamber | Sugar cane molasses | Modeling based on kinetic approach; 4–5.33 kPa; recycling of liquid stream from flash; cell recycling; 98% conversion; 23–26.7 g/(L h) productivity | Costa et al. (2001), da Silva et al. (1999b) |
| Conjugated | Batch fermentation coupled with distillation | Immobilized *S. cerevisiae* in Ca alginate/distillation | Glucose-containing medium | Distillation was carried out periodically; recycling of distillation bottoms; 500 h of cultivation; yield 92%; EtOH conc. 10–80 g/L; reduced wastewater | Kishimoto et al. (1997) |
| Conjugated | SCMR coupled with distillation | Free or immobilized *S. cerevisiae*/distillation | Glucose-containing medium | High cellular retention by ceramic membrane; recycling of distillation bottoms; 100 h of cultivation; without wastewater; productivity 13.1–14.5 g/(L h); EtOH conc. 20–50 g/L | Ohashi et al. (1998) |
| Conjugated | Continuous fermentation coupled with stripping | *S. cerevisiae*/ethanol stripping with $CO_2$ | Glucose-containing medium/saccharified corn mash | Fermenter coupled with a packed column; 60–185 d of operation; yield 0.48–0.50 g/g; EtOH conc. 55.8–64.4 g/L in fermenter and 257–364 g/L in condensate; productivity 7.5–15.8 g/(L h) | Taylor et al. (1996, 1998, 2000a) |
| Conjugated | Continuous fermentation coupled with filtration | Inhibitor-tolerant *S. cerevisiae*/cross-flow microfiltration unit with stirring | Undetoxified dilute acid spruce hydrolyzate | Supplementation with complete mineral medium; 90% cell recirculation; microaerobic cond.; productivity up to 1.44 g/(L h); 96 h of operation | Brandberg et al. (2005) |
| Simultaneous | Continuous membrane –filtration bioreactor | *S. cerevisiae*/internal ceramic tubes inside the fermentor | Wood hydrolyzate | High cell retention; EtOH conc. 58.8–76.9 g/L; yield 0.43 g/g; productivity 12.9–16.9 g/(L h); 55 h of operation | Lee et al. (2000) |
| Conjugated | Batch fermentation coupled with continuous perstraction | *S. bayanus*/Teflon sheet soaked with isotridecanol | Glucose-containing medium | Water was used as an extractant; EtOH conc. in the broth 75–61 g/L, in the extractant 38 g/L; yield 0.46; aver. productivity 1.2 g/(L h) | Christen et al. (1990) |
| Conjugated | Continuous fermentation coupled with continuous perstraction | Immobilized *S. cerevisiae* in Na alginate/membrane of the type of artificial kidneys | Glucose-containing medium | Tri-*n*-butylphosphate was used as an extractant; glucose feed concn. 506 g/L; aver. EtOH conc. in broth 67 g/L, in the extractant 53 g/L; productivity 48 g/(L h); up to 430 h of operation | Matsumura and Märkl (1986) |
| Conjugated | Batch fermentation coupled with continuous pervaporation | *S. cerevisiae*/silicalite zeolite membrane | Glucose-containing medium | For 4.6 wt.% EtOH in the broth, EtOH in permeate reaches 81.7 wt.%; separation factor of membrane 88; up to 48 h of operation | Nomura et al. (2002) |
| Conjugated | Batch co-fermentation coupled with continuous pervaporation | *P. stipitis*/polytetrafluoro-ethylen membrane | Enzymatically saccharified exploded rice straw | For 10 g/L EtOH in the broth, EtOH in permeate reaches 50 g/L; yield 0.43 g/g; 100 h of operation | Nakamura et al. (2001) |
| Conjugated | Fed-batch fermentation coupled with pervaporation | Immobilized *S. cerevisiae* in Ca alginate/microporous polypropylene membrane | | 72 h cultivation; EtOH conc. 50 g/L; yield 0.49 g/g; productivity 2.9 g/(L h); 61.5% reduction in wastewater | Kaseno et al. (1998) |

| | | | | | |
|---|---|---|---|---|---|
| Conjugated | Continuous fermentation coupled with pervaporation | *S. cerevisiae*/commercial polydimetylsiloxane membranes | Starch from dry milling plant | Aspen Plus simulation based on fermentation-pervaporation lab experiments; EtOH conc. in permeate 420 g/L; recycling of retentate to fermenter, reduction of cost associated with fermentation by 75% | O'Brien et al. (2000) |
| | | Immobilized *S. cerevisiae* on beads of PAAH gel coated with Ca alginate/membrane of silicone composite on a polysulfone support | Glucose/molasses | For 4 wt.% EtOH conc. in the broth, EtOH in permeate reaches 12–20% wt.%; yield 0.36–0.41 g/g; productivity 20–30 g/(L h); over 40 d of operation | Shabtai et al. (1991) |
| Conjugated | Continuous SSF coupled with pervaporation | *S. cerevisiae* + *T. reesei* cellulases/silicate membrane | Cellulose | Modeling based on kinetic approach; yield 0.44 g/g; EtOH conc. 248.3 g/L in permeate and 4.1 g/L in broth; reduced product inhibition effect; residence time of 72 h; 60–99% substrate conversion | Sánchez et al. (2005) |
| Conjugated | Batch fermentation coupled with membrane distillation | *S. cerevisiae*/capillary polypropylene membrane | Sucrose-containing medium | 2–3 d cultivation; periodic flow of broth through membrane distillation module during 5–6 h per day or continuous coupling to bioreactor; yield 0.47–0.51 g/g; EtOH conc. 50 g/L in broth; productivity 2.5–5.5 g/(L h) | Gryta (2001, 2002), Gryta et al. (2000) |
| Conjugated | Continuous fermentation coupled with membrane distillation | *S. cerevisiae* and *S. uvarum*/polypropylene and poly(tetrafluoro-ethylene) membranes | Glucose and molasses solutions | 430–695 h cultivation; EtOH conc. 60 g/L in broth and 200–400 g/L in cold trap; high concentrated medium (316 g/L molasses) | Calibo et al. (1989) |
| Simultaneous | ALSA | *S. uvarum*/ethanol stripping with $CO_2$ | Glucose | Fed-batch regime; EtOH conc. >130 g/L within 24 h | Gong et al. (1999) |
| Simultaneous | MSCRS | *Kluyveromyces marxianus*/*Pichia stipitis*/ethanol stripping with $CO_2$ | Lignocellulosic biomass (oat hulls) | 6-stage reactor separator; SSF of cellulose in first 3 stages with *K. marxianus*; xylose fermentation in last 3 stages with *P. stipitis*; recycle of broth | Dale and Moelhman (2001) |
| | MSCRS | *S. cerevisiae* or *Zymomonas mobilis*/ethanol stripping with $CO_2$ | Gelatinized starch | 4-stage reactor separator; enriching and stripping sections; SSF of starch; ethanol stripping | Dale (1992) |
| Conjugated | Continuous fermentation coupled with liquid–liquid extraction | Immobilized yeast/*n*-dodecanol | Glucose-containing medium | 18 d of operation; use of very concentrated feedstocks (10–48% w/w); 78% reduction of aqueous effluents | Gyamerah and Glover (1996) |
| Simultaneous | Batch extractive co-fermentation | *Z. mobilis*/*n*-dodecanol | Glucose and xylose | Modeling based on kinetic approach and liquid–liquid equilibrium; solvent is regenerated by flashing; productivity 2.2–3.0 g/(L h); solvent volume/aqueous volume ratio 1.33–3.0 | Gutiérrez et al. (2005) |
| Simultaneous | Continuous extractive fermentation | Immobilized *S. cerevisiae*/*n*-dodecanol | Glucose | Pneumatically pulsed packed reactor; flowrates: solvent 1-2.55 L/h, medium 0.057–0.073 L/h; feed glucose conc. 261–409 g/L; EtOH conc. in solvent 3.37–10 g/L, in broth 9.4–33 g/L; yield 0.51; productivity 1.03 g/(L h) | Minier and Goma (1982) |
| | | *Clostridium thermohydrosulfuricum*/oleyl alcohol | Glucose | Flowrates: broth 0.15–0.55 L/h, solvent 0–18 L/h; feed glucose conc. 12.5–100 g/L; EtOH conc. in the broth <4.47 g/L, in the reextraction water 3–14 g/L; 65 °C; productivity <0.128 g/(L h) | Weilnhammer and Blass (1994) |
| Simultaneous | Fed-batch SSEF | *S. cerevisiae*/commercial cellulases/oleyl alcohol | Primary clarifier sludge from chemical pulping process/cellulose | Reactor with up to 2.5% aqueous phase; 50% substrate conversion; 48–275 h cultivation; 65% increase in productivity compared to conventional fed-batch process | Moritz and Duff (1996) |

(*continued on next page*)

Table 3 (*continued*)

| Type of intensification process[a] | Technology | Bioagent/unit operation features | Feedstock/medium | Remarks | References |
|---|---|---|---|---|---|
| Simultaneous | HFMEF | *S. cerevisiae*/hydrophobic microporous hollow fibers/oleyl alcohol or dibutyl phtalate | Glucose | Yeast cells are immobilized on the shell side; solvent flows in fiber lumen; feed glucose conc. 300 g/L; productivity 31.6 g/(L h) | Kang et al. (1990) |
| Simultaneous-conjugated | CMFS | *S. cerevisiae*/membrane bioreactor with continuous removal of ethanol by pervaporation/coupling with cell separator | Not specified | Modeling study; higher dilution rates and productivity (up to 13.5 g/(L h)); recycle ratio 0–2.0; pervaporation factor 0–2.5 $h^{-1}$; EtOH conc. 10–47 g/L; cell conc. increased from 1.9 to 14.6 g/L due to recycle and pervaporation. | Kargupta et al. (1998) |

ALSA, air-lift reactor with a side arm; CMFS, continuous membrane fermentor–separator; HFME, hollow-fiber membrane extractive fermentor; MSCRS, multi-stage continuous reactor–separator; PAAH, polyacrylamide hydrazide; SCMR, stirred ceramic membrane reactor; SSEF, simultaneous saccharification and extractive fermentation.

[a] According to the classification of Rivera and Cardona (2004).

exogenous enzymes, and the utilization of only one bioagent during the transformation, the yeast. In this way, a single microorganism can directly convert the starch into ethanol (CBP process, see Fig. 3). The savings obtained during the commercial implementation of such a process could offset by far the lower growth rates and the longer fermentation times. Lynd et al. (2002) mention, among the saccharolytic genes that have been introduced into microorganisms as *S. cerevisiae* and *K. oxytoca*, those encoding α-amylase, glucoamylase, amylopullulanase, pectate lyase and polygalacturonase, obtained from bacterial and fungal sources. The processes with microorganisms modified by genetic engineering involve the optimization not only of microbial physiology parameters, but also of cell culture parameters (retention and stability of plasmids, nutritional factors, cell growth, and protein synthesis). Therefore, the modeling of these processes and the application of the principles of biochemical engineering can be helpful considering the uncertainties and complexities inherent to these biological systems. An example of this type of modeling is the work of Kobayashi and Nakamura (2004) that corroborated experimentally at laboratory scale the higher productivity of the continuous fermentation process using starch-degrading recombinant yeast cells immobilized in calcium alginate beads in comparison to the free cell system.

## 5. Reaction–separation integration

Above-mentioned types of integration allow the increase of process efficiency through the improvement of reaction processes. However, separation is the step where major costs are generated in process industry. Therefore, reaction–separation integration could have the highest impact on the overall process in comparison with homogeneous integration of processes (reaction–reaction, separation–separation). This has been demonstrated in the case of reactive distillation processes (Pisarenko et al., 1999, 2001), particularly, in the lactic acid recovery by reactive distillation (Cardona et al., 2004), and in the production of allyl alcohol by allyl acetate butanolysis (Anokhina et al., 1996a, 1996b). Hence, the integration of reaction–separation processes plays a very important role in the production of fuel ethanol. Most of integrated schemes of this type are oriented to the integration between fermentation step and several kinds of separation unit operations, although some proposals have been formulated for other steps of global process.

### 5.1. Integration of enzymatic hydrolysis and separation

For the case of starch hydrolysis, the utilization of immobilized enzymes and hollow-fiber membrane reactors is considered as a promising alternative. Mandavilli (2000) immobilized glucoamylase on a cellulose support for hydrolysis of potatoes starch in a fixed-bed bioreactor; these authors studied and modeled the mass transfer and

enzymatic kinetics occurring within the reactor. López-Ulibarri and Hall (1997) optimized a reactor of this type that employed cassava flour previously gelatinized by extrusion and *Aspergillus niger* glucoamylase. This system allowed the production of glucose solutions of about 10% (w/v) without the need of a preceding liquefaction step, which could improve the economy of the process. In addition, the enzymes are confined inside the reactor allowing the separation of the substrate and the product (glucose) and making possible the reutilization of the enzymes while preserving their activity as free catalysts. This kind of configuration is very important when macromolecules as starch are hydrolyzed and it represents an effective type of reaction–separation integration process. This approach has also been applied to the saccharification of lignocellulosic biomass using commercial *T. reesei* cellulases attaining an increase of 53% in substrate conversion ratio in comparison to 35% conversion in the case of traditional batch operations. This increase was mainly due to the reduction in the inhibition effect of formed sugars on cellulases and to the increase in productivity during continuous operation (Gan et al., 2002).

### 5.2. Ethanol removal from culture broth

The reaction–separation integration is particularly an attractive alternative for the intensification of alcoholic fermentation processes. When ethanol is removed from the culture broth, its inhibition effect on growth rate is diminished or neutralized. The importance of this fact has been recognized in such early works as the one presented by Maiorella et al. (1984) where membrane, extractive and flashing methods for ethanol removal were assessed for ethanol production from molasses and cellulose hydrolyzate. For this reason, most of the proposed configurations using this type of integration are related to the ethanol removal by different means including the coupling of different unit operations to the fermentation, or the realization of simultaneous processes for favoring the *in situ* removal of ethanol from culture broth. Some examples of such type of reaction–separation integration processes can be found in Table 3.

#### 5.2.1. Ethanol removal by vacuum

The coupling of fermentor vessel with a vacuum chamber extracting the more volatile ethanol from fermentation broth allows the partial product removal and the increase of overall process productivity. Cysewski and Wilke (1977) showed the viability of operating a vacuum fermentor with cell recycle in a continuous regime using *S. cerevisiae* and a glucose-based medium. A 12-fold increase in ethanol productivities could be attained (see Table 3). However the system required the addition of pure oxygen and the implementation of a bleed stream of broth. In general, the research on vacuum fermentation has received less attention than other forms of ethanol removal. Nevertheless, da Silva et al. (1999b) point out that vacuum fermentation using a flash chamber coupled to the bioreactor can demonstrate better technical indexes than extractive fermentation or fermentation coupled to pervaporation (see Fig. 5a). Ishida and Shimizu (1996) proposed a novel regime for carrying out the repeated-batch alcoholic fermentation coupled with batch distillation obtaining ethanol concentrations of 400 g/L.

#### 5.2.2. Ethanol removal by gas stripping

Ethanol can be removed from the culture broth through gas stripping that makes possible the increase in the concentration of sugars in the stream feeding the fermentor. Taylor et al. (1998) studied this integrated process in the case of dry-milling ethanol process in a pilot plant coupling a 30 L fermentor with a 10 cm packed column for ethanol removal by the $CO_2$ (stripping gas) released during the fermentation. A simplified scheme of the proposed configuration is shown in Fig. 5b. The proposed model showed that ethanol inhibition influences especially the cell yield obtaining a value of 60 g/L of ethanol in the broth above which the inhibition is very strong. The authors point out that the values of kinetic parameters depend in a high degree on the type of fermentation: batch or continuous. Later, Taylor et al. (2000a) utilized a saccharified corn mash containing high levels of suspended solids as a feed and compared the results obtained with a state-of-the-art dry-milling process using Aspen Plus. Savings of 0.8 US cents/L of ethanol can be attained in comparison with the state-of-the-art process for which saccharification and fermentation are carried out separately.

Gong et al. (1999) report the simultaneous variant of the fermentation-stripping process using an air-lift reactor with a side arm (external loop) that improves liquid circulation and mass transfer (see Table 3). A more complex configuration integrating the fermentation and stripping was developed by the company Bio-Process Innovation, Inc. (USA) that designed and built a pilot plant for ethanol production from lignocellulosic biomass using a 130 L multistage continuous stirred reactor separator (MSCRS) for the SSF of cellulose and hemicellulose (Dale and Moelhman, 2001). The MSCRS consists of a series of six stirred stages in which the SSF of biomass is carried out. Each stage has a stirred tank for the reaction and a gas–liquid separation contactor. In the three upper stages, SSF of cellulose is carried out at 42 °C using a thermotolerant *K. marxianus*, while in three lower stages, the fermentation of xylose is achieved using the yeast *P. stipitis* at 30 °C. In addition, part of the broth containing enzymes is recirculated from the last stage to the first upper stage in order to favor the reaction with the fresh pretreated biomass. Reaction–reaction integration is implemented considering that commercial cellulases were added for the saccharification of pretreated biomass; this defines the process temperature helping the generation of ethanol vapors. The broth overflowing from one stage into the next stage is contacted with a stripping stream of $CO_2$ that entraps the ethanol. Gas stream passes across the reactor and then through

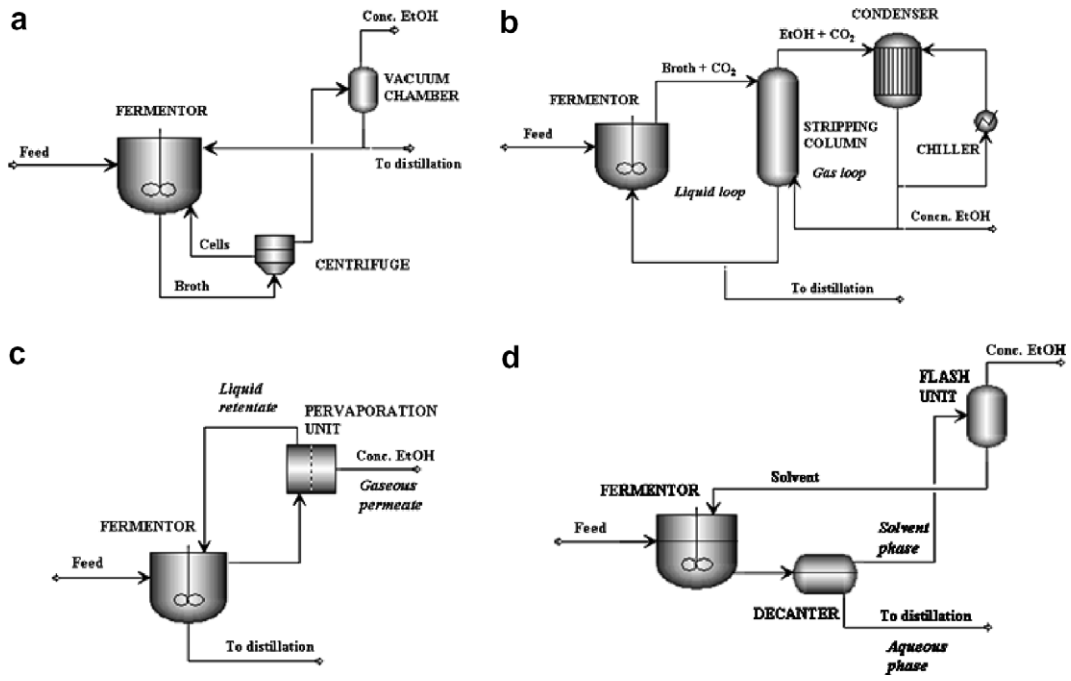


Fig. 5. Simplified diagrams corresponding to some configurations for ethanol removal from culture broth. (a) Vacuum fermentation with cell recycling. (b) Fermentation coupled with gas stripping. (c) Fermentation coupled with pervaporation. (d) Extractive fermentation.

an absorption tower where is contacted with water for removing ethanol vapors. $CO_2$ is again recirculated to the reactor. In this way, reaction–separation integration is verified through the *in situ* removal of ethanol produced in each stage. The same company has installed a pilot plant reactor using MSCRS technology (Dale, 1992) in the plant of Permeate Refining, Inc., located in Iowa (USA) that produces 11.36 mill liters per year of ethanol from starch. This unit has been in operation since September 1995 and utilizes starch dextrines, thought its use for lignocellulosic biomass has been proposed. Unfortunately, no reports are available describing the modeling and performance of this type of configurations.

### 5.2.3. Ethanol removal by membranes

Some laboratory configurations of this type have shown interesting results, but their implementation at industrial scale can be very difficult. The utilization of ceramic membranes has been proposed for the filtration of cell biomass and the removal of ethanol during the fermentation (Ohashi et al., 1998). The removed ethanol is distilled and the obtained bottoms are recycled to the culture broth resulting in a drastic reduction of generated wastewater. This configuration uses a Stirred Ceramic Membrane Reactor (SCMR). In the same way, immobilized cells can be utilized in order to allow an easier separation of ethanol and the recirculation of distillation bottoms to the reactor (Kishimoto et al., 1997). Kobayashi et al. (1995) developed a mathematical model for the optimization of temperature profiling during the batch operation of a fermentor coupled with a hollow-fiber module; the temperature was kept initially at 30 °C descending later to 20 °C and attaining higher ethanol concentration and productivity. However, it is necessary to analyze the scalability of these configurations due to their complexities (immobilization, presence of membranes, recirculation, repeated batches). The utilization of liquid membranes (porous material with an organic liquid) in schemes involving the extraction of ethanol by the organic phase and the reextraction with a liquid stripping phase used as an extractant (perstraction or membrane-aided solvent extraction) or gaseous stripping phase (pervaporation) have been also coupled to the fermentation process showing the increased effectiveness of the latter configuration (Christen et al., 1990; see Table 3).

Pervaporation has offered new possibilities for integration. The coupling of fermentation with the pervaporation allows the removing of produced ethanol, reducing the natural inhibition of the cell growth caused by high concentrations of ethyl alcohol (see Fig. 5c). Nomura et al. (2002) observed that separation factor of silicalite zeolite membranes used for continuous pervaporation of fermentation broth was higher than corresponding value for ethanol–water mixtures due to the presence of salts that enhance the ethanol selectivity. Ikegami et al. (2003, 2004) utilized this same kind of membrane coated with two types of silicone rubber or covered with a silicone rubber sheet as a hydrophobic material for obtaining concentrated solutions of ethanol. The coupling of *C. thermohydrosulfuricum* that directly converts uncooked starch into ethanol with pervaporation has also been tested obtaining ethanol concentrations in the permeate of 27–32% w/w (Mori and Inaba, 1990).

O'Brien et al. (2000) utilized process simulation tools (Aspen Plus) for evaluating the costs of the global process

involving fermentation–pervaporation in comparison to the conventional batch process from starch. Fermentation–pervaporation was simulated based on experimental data from tests carried out during more than 200 h employing commercial membranes of polydimethylsiloxane. Performed simulations showed costs slightly higher for the coupled fermentation–pervaporation process due to the capital and membrane costs. Nevertheless, fermentation costs were reduced in 75% and distillation costs decreased significantly. Sensitivity analysis indicated that few improvements in membrane flux or selectivity could make competitive this integrated process. Wu et al. (2005) have investigated the mass transfer coefficients for this type of membrane in the case of pervaporation of fermentation broths showing that active yeast cells were favorable for ethanol recovery.

Sánchez et al. (2005) carried out the modeling of SSF of lignocellulosic biomass coupled with a pervaporation unit. Ethanol removal allowed the reduction of the inhibition effect on cell growth by the accumulation of ethanol in the medium, whereas the SSF process permitted the reduction of the inhibition by glucose and cellobiose experimented by the enzymes during the cellulose hydrolysis. This reaction–reaction–separation integration configuration demonstrated the possibility of reaching higher productivities for the continuous process as well as the production of permeates with an elevated ethanol concentration in comparison to the broth; this implies the reduction in energetic costs during subsequent distillation. Kargupta et al. (1998) carried out the simulation of continuous membrane fermentor–separator (CMFS) removing ethanol by pervaporation in a membrane reactor, which is coupled with a cell separator in order to increase the concentration of cells inside the reactor by recycling them. The proposed models predict an increase in productivity because this system could be operated at high dilution rates as a consequence of *in situ* product removal and higher cell concentrations.

Besides pervaporation, membrane distillation has been studied. In this type of distillation, aqueous solution is heated for the formation of vapors, which go through a hydrophobic porous membrane favoring the pass of vapors of ethanol (that is more volatile) related to the vapors of water. The process driving force is the gradient of partial pressures mainly caused by the difference of temperatures across the membrane. Gryta et al. (2000) implemented a batch fermentor coupled with a membrane distillation module leading to the ethanol removal from culture broth diminishing the inhibition effect and obtaining an increase in ethanol yield and productivities (see Table 3). Gryta (2001) points out that when tubular fermentor working in continuous regime is coupled with the membrane distillation module, higher increases in ethanol productivity can be achieved (up to 5.5 g/(L h)). This author determined that the number of yeast cells that are deposited on the membrane is practically zero during the operation of these modules (Gryta, 2002). Calibo et al. (1989) also demonstrated the possibility of coupling the continuous fermentation with membrane distillation. They used a column fermentor, a cell settler and a membrane module. This system operated during almost 700 h with a feed of molasses. García-Payo et al. (2000) studied the influence of different parameters for the case of air gap membrane distillation based on the model of temperature polarization. It was observed that permeate flux increases in a quadratic way when ethanol concentration increases in the membrane distillation module. Similarly, Banat and Simandl (1999) indicate that the effects of concentration and temperature polarization should be accounted during the modeling of this process and highlight the need of optimizing it with respect to feed stream temperature. Banat et al. (1999) also analyzed different models based on the Fick's law and on the solution of Maxwell–Stefan equations for this type of distillation. Likewise, the characteristics of the vacuum membrane distillation (Izquierdo-Gil and Jonsson, 2003) and direct contact membrane distillation have been studied for the concentration of aqueous solutions of ethanol (Fujii et al., 1992a,b). Without doubt, these studies are of great interest considering the simulation of these integrated configurations.

In the case of the bioethanol production from sugar cane, the integration of fermentation with pervaporation or vacuum membrane distillation allows the recovery of a valuable product, the fructose. For this, mutant strains of yeasts without the capacity of assimilating this monosaccharide should be utilized. Thus, continuous ethanol removal through the membranes coupled to the fermentor makes possible the accumulation of fructose in the culture medium that can be recovered in an extraction column. According to Di Luccio et al. (2002), the simulation of this process based on experimental data and semiempiric models for the evaluation of the required membranes area allowed performing a preliminary economic analysis. This analysis showed that variable costs involving membranes area influence in a higher degree the viability of the process. The process only is viable if the costs of membranes is not greater than US$550/m$^2$ for a new plant or US$800/m$^2$ for an adapted plant considering an internal return rate of 17%.

#### 5.2.4. Ethanol removal by liquid extraction

A reasonable approach for increasing the productivity of alcoholic fermentation is the removal of the product that causes the inhibition through an extractive biocompatible agent (solvent) that favors the migration of ethanol to solvent phase, process known as extractive fermentation. Minier and Goma (1982) showed that primary aliphatic alcohols with a chain length having less than twelve carbons inhibit the growth of yeast cells. They chose the fatty alcohol *n*-dodecanol as a solvent for *in-situ* extraction of ethanol in an especial continuous pulse packed column with immobilized cells of *S. cerevisiae*. This configuration allowed the utilization of very concentrated glucose feed

due to the reduction of ethanol in the culture broth. In addition, immobilization seems to protect the cells against solvent toxicity (Aires Barros et al., 1987; Tanaka et al., 1987).

Gyamerah and Glover (1996) implemented a process where the fermentation stage was coupled with an apparatus for liquid–liquid extraction in a continuous regime at pilot-scale level. They chose *n*-dodecanol by its high very low toxicity for ethanol-producing microorganisms. However, this solvent has some drawbacks: it tends to form a stable emulsion with the culture broth, its melting point is relatively high (26 °C) considering fermentation conditions, and its distribution coefficient related to water is not very high (Kollerup and Daugulis, 1985). In addition, Kirbaşlar et al. (2001) experimentally showed that small amounts of water migrate to *n*-dodecanol in water–ethanol–*n*-dodecanol ternary systems. Weilnhammer and Blass (1994) proposed a simple model based on the mass balance of different components for the description of extractive fermentation with *C. thermohydrosulfuricum* using oleyl alcohol as a solvent; this model allowed the evaluation of the economy of the process with and without solvent based on production costs. Kollerup and Daugulis (1985) proposed a mathematical model for describing extractive fermentation process for continuous production of ethanol from glucose-containing medium. In this model, a simple relationship between ethanol concentration in aqueous phase and ethanol content in solvent phase was considered. Additionally, kinetic description of microbial growth did not take into account the inhibition effect due to high concentrations of substrate. Fournier (1986) developed a rigorous description considering the use of UNIFAC equations for continuous extractive fermentation. Gutiérrez et al. (2005) modeled the batch extractive co-fermentation from pretreated hydrolyzed lignocellulosic biomass coupling the equations representing the kinetics of the biological process with the equations describing the liquid–liquid equilibrium using activity models as NRTL, UNIFAC and UNIQUAC. Liquid medium from this bioreactor is continuously removed in order to separate the cells and carry out the decantation of both phases. Aqueous phase is recycled back to the bioreactor and ethanol-rich solvent phase is flashed for the regeneration of solvent and the production of almost pure ethanol (a simplified scheme of this configuration is shown in Fig. 5d). Performed analysis involving the comparison with conventional configurations from energy point of view demonstrated the possibility of reducing energy consumption and increasing product yield. These authors also proposed the application of a short-cut method based on thermodynamic-topological analysis for evaluating the work regime of extractive fermentation; in this way, the space of initial operating conditions can be narrowed for performing rigorous modeling (Sánchez et al., 2006a). In addition, this short-cut method can offer valuable information for optimization of such operating variables as the dilution rate and the ratio of aqueous phase flow rate to solvent flow rate in the feed of the reactor. This approach was applied to the modeling of alcoholic extractive fermentation with *n*-dodecanol using GAMS software for optimization procedures.

Further integration could include a process where simultaneous saccharification and extractive fermentation could be carried out, as reported by Moritz and Duff (1996). These authors used oleyl alcohol and demonstrated that this solvent had effect neither on cellulases, nor cell biomass, i.e., it was biocompatible for both biological agents involved in the process. Kang et al. (1990) utilized a hollow-fiber membrane reactor for carrying out extractive fermentation with yeast using oleyl alcohol and dibutyl phthalate obtaining productivities of 31.6 g/(L h). Fournier (1988) proposed a mathematical model for this type of hollow-fiber membrane extractive fermentors that predicts significant improvements in productivity related to conventional CSTR with solvent extraction. The utilization of a liquid-lift external loop bioreactor where the solvent is sparged into the base of the column containing a second liquid (the broth) of higher density has been proposed for the extraction of ethanol as well (Modaressi et al., 1997; Stang et al., 2001).

Eldridge et al. (1989) carried out several biompatibility and extraction tests for the removal of ethanol from culture broth using a mixture of a commercial parafinic solvent (70 wt.%) and tributyl phosphate (30 wt.%); these authors also applied several mass transfer model to the obtained results. Oliveira et al. (1998) and Oliveira et al. (2001) proposed an extractive biocatalytic process in which ethanol produced by yeasts is extracted by oleic acid and used as substrate for lipase-catalyzed esterification reaction with this same acid. In this way, the combination of the enzymatic reaction and the extractive fermentation in a single vessel improves the product extraction. Acceptable results for broths with a high concentration of glucose (300 g/L) were obtained, although experiments without lipase (only extractive fermentation) indicate that oleic acid is not a good extracting agent for ethanol since its concentration in aqueous phase was higher than in solvent phase at the end of fermentation. L'Italien et al. (1989) proposed and tested a regime of fermentation using as a solvent supercritical carbon dioxide; for this, it was necessary to organize a cyclic process with periods of high-cell atmospheric fermentation followed by a period of hyperbaric conditions (7 MPa) for the rapid extraction of ethanol by $CO_2$; however, the complexity of the process and the loss of viability of the cells during prolonged intervals under high pressure makes this technology non-viable to date. For the extractive fermentation process, the presence of microbial cells can reduce the rate of ethanol extraction. Crabbe et al. (1986) found that the yeast cells severely decreased the rate of extraction employing *n*-decanol as a solvent; the authors assumed that the studied effects can be extrapolated to *n*-dodecanol.

The selection of the solvent is a crucial factor for extractive fermentation technology. From modeling studies, Tsuji et al. (1986) showed that a high distribution coefficient of ethanol between the solvent and aqueous phases (high solvent selectivity) allows increasing both the productivity and the product concentration. In addition, the solvent should be no toxic for fermenting cells. Bruce and Daugulis (1991) developed a solvent screening program for evaluating possible extracting agents to be used in extractive fermentation configurations. This program considered the biocompatibility of the solvent and utilized UNIFAC activity model for predicting liquid–liquid equilibrium data. Using this program, these authors (Bruce and Daugulis, 1992) identified the solvent mixture of oleyl alcohol with 5% (v/v) 4-heptanone as a promising extractant due to its reduced inhibitory effect and increased distribution coefficient. A large amount of alcohols have been tested in order to examine their ethanol extracting properties in water–ethanol–solvent systems (Offeman et al., 2005a,b). The collected data have allowed gaining insight about the relationship between the structure of the solvent and its extracting characteristics; for this type of work, molecular simulation could provide a deeper understanding on solvent conformation and associations among water, ethanol, and solvent. Although these works are intended to the selection of proper solvent for ethanol dehydration, the obtained results are of great value for extractive fermentation studies provided the needed biocompatibility tests.

Other approach for extractive fermentation that has been used is the application of aqueous two-phase fermentation where two phases are formed in the bioreactor as a result of adding two or more incompatible polymers (Banik et al., 2003). In this way, ethanol can be partitioned between both phases accumulating in the upper layer whereas cell biomass is accumulated in the lower phase; this allows the separation of the ethanol-rich phase in order to distillate the alcohol and reduce the inhibition effect (Kühn, 1980). Nevertheless, the complexity of the process in the case of continuous regime and the high cost of polymers have caused that this technology has not been further developed for ethanol production.

The enhancement of the efficiency of fuel ethanol production processes requires the reduction of high costs related to the separation of ethanol from dilute alcoholic solutions. The product removal from the culture broth during the fermentation process not only significantly increases the concentration of ethanol in the outlet streams, but also improves the cultivation process by diminishing the negative effect caused by the product inhibition that affects cell growth rate and ethanol production. The further integration of processes like SSF or SSCF with procedures for ethanol removing will boost both the biological conversion and ethanol recovery thanks to the intensification caused by the change of the concentrational field and by the neutralization of different inhibitory effects generated by substrates and products.

## 6. Separation–separation integration

The development of technologies for separation–separation integration has been linked to the development of the different involved unit operations and to new approaches for process intensification. The examples of separation–separation integration in the case of ethanol production mostly correspond to integration of the conjugated type, i.e., when integrated processes are carried out in different equipments closing the flowsheet by fluxes or refluxes. In this context, the application of saline extractive distillation and membrane technologies for the integration of several configurations for separation and dehydration of ethanol should be highlighted.

Integration possibilities are particularly important for ethanol dehydration. In the case of saline extractive distillation, Llano-Restrepo and Aguilar-Arias (2003) analyzed the flowsheet proposed by Barba et al. (1985) that includes preconcentration, saline extractive distillation using a column operating at 0.25 atm, crystallization by vacuum evaporation and spray drying for recovery of anhydrous salt. Cited results indicate that energy costs of saline distillation were lower than in the case of azeotropic distillation (using benzene, pentane or diethyl ether), extractive distillation (using ethylene glycol or gasoline) or solvent extraction, being almost the same than the costs of pervaporation. Pinto et al. (2000) employ Aspen Plus for the simulation and optimization of the saline extractive distillation for several substances (NaCl, KCl, KI and $CaCl_2$) applying the NRTL model developed for electrolytic systems to calculate activity coefficients. This configuration was compared to the simulated scheme of conventional extractive distillation with ethylene glycol and with data for azeotropic distillation. Obtained results show considerably lower energy consumption for the process with salts. However, for this latter case, the recovery of salts was not simulated. Thus, if evaporation and recrystallization of salts is contemplated, energy requirements could significantly increase taking into account the energetic expenditures of salts recovery.

In this way, the utilization of commercial simulators shows the viability for predicting the behavior of a given process configuration provided the appropriate thermodynamic models of studied systems. Montoya et al. (2005) carried out the simulation of the ethanol dehydration process complemented with the evaluation of energetic and capital costs. Through proper methodologies based on the topological thermodynamics, the synthesis of related distillation trains and the evaluation of the separability of different components involved in culture broths from which ethanol should be recovered were performed. The results showed that dehydration with molecular sieves presented lower operation costs of all the analyzed flowsheets, though its capital costs are higher due to the complexity of automation and control system inherent to the pressure swing adsorption technology.

Gros et al. (1998) describe the process synthesis for ethanol dehydration using near critical propane. To this end,

these authors combined thermodynamic models for the description of ethanol recovery under supercritical conditions based on Group Contribution-Associating-Equation of State (GCA-EOS) with robust methods of simulation and optimization (integrating the SQP with MINLP). Considering the energy consumption as the objective function, the developed software analyzed the main units required by the configuration: high-pressure multistage extractors, distillation columns and multiphase flash separators. Obtained results showed that configurations involving vapor recompression and feed preconcentration, are competitive alternatives in comparison to azeotropic distillation.

The utilization of pervaporation for the production of absolute (anhydrous) ethanol through its coupling with the previous distillation step has been reported. The modeling and optimization of the process using MINLP tools showed 12% savings in the production costs considering a 32% increase in membrane area and the reduction in both reflux ratio and ethanol concentration in the distillate of the column (Lelkes et al., 2000; Szitkai et al., 2002). Through pilot-plant studies, the integration of distillation process with the pervaporation has been achieved resulting in good indexes in terms of energy savings. These savings are due to the low operation costs of pervaporation and to the high yield of dehydrated ethanol, typical of pervaporation processes (Tsuyomoto et al., 1997). The comparison between azeotropic distillation using benzene and pervaporation system using multiple membrane modules showed that, at the same ethanol production rate and quality (99.8 wt.%), operation costs, including the membrane replacement every 2–4 years, are approximately 1/3–1/4 of those of azeotropic distillation.

## 7. Energy integration

Described types of integration are related to the integration of material flows either for their transformation or for the separation of their components. Similarly, the energy integration of the different steps for ethanol productions is possible. Energy integration, particularly heat integration, looks for the best utilization of energy flows (heat, mechanical and electrical) generated or consumed inside the process with the aim of reducing the consumption of external sources of energy as electricity, fossil fuels (oil, natural gas) mainly used for steam generation, and cooling water. Pinch technology is one of the most widely applied approaches for heat integration in process industry, especially in petrochemical industry. This technology provides the necessary tools for design of heat exchanger networks including plant utilities. During preliminary design of the heat exchange network, pinch technology allows obtaining the best values of many process parameters as the type of utilities and their specifications, minimum number of heat exchange units and their transfer areas, as well as the estimation of capital and operation costs of these units. These values can be obtained without the necessity of performing the detailed design of the heat exchanger network. Only the thermal data of process streams are required (Shenoy, 1995). In this way, heat integration has become one of the most important techniques for process integration during the synthesis of diverse technological flowsheets.

Ficarella and Laforgia (1999) applied the pinch technology based on the procedures described by Linnhoff et al. (1991) for the separation step of a distillery producing ethanol (96%) from low quality wine and wine dregs. Through the analysis of involved streams for a plant operating in Italy and classifying them as sources or sinks, the use of the so-called heat pumps, equipments converting the available heat in the process in order to utilize it within the same process or even outside it, was suggested. Among the proposed heat pumps, cogeneration systems of mechanical energy and heat for steam-turbine-driven compressors were proposed; for these systems, the bottoms from distillation columns or steam condensates were chosen as working fluids. For the case of ethanol production from molasses, the process flowsheet was simulated and optimized by heat integration, especially in the separation step by distillation (Sobočan and Glavič, 2000). The simulation was made by short-cut and rigorous methods in order to perform the heat integration. For ethanol concentrations up to 95.7 wt.%, the optimal configuration corresponds to one single column and not two, as had been proposed. This work demonstrates the usefulness of heat integration since the optimal design showed a 27% reduction in the total costs. Grisales et al. (2005) utilized the heat integration approach for the analysis of fermentation, distillation and evaporation steps of fuel ethanol production process form lignocellulosic biomass using azeotropic distillation for ethanol dehydration. Aspen Plus was utilized for the preliminary balances of mass and energy. By a graphic representation of the energy requirements of the process, the possibilities for heat exchange between hot and cold streams were identified employing the rules of pinch technology. This methodology allowed the design of a more optimal heat exchanger network that implied the reduction in the use of utilities (steam and cooling water) with the corresponding reduction in operation costs.

Heat integration can be done within a same unit. One of the applications of this kind of integration is the secondary reflux and vaporization (SRV) distillation in which reflux and vaporization rates are deliberately manipulated to enhance the overall thermal efficiency. This manipulation is accomplished by heat exchange between the rectifying and stripping sections of the distillation column. In order to guarantee a temperature difference that favors heat exchange, rectifying section is operated at higher pressures than those of the stripping section. In this way, the liquid reflux rate steadily increases as one proceeds down the rectifying section, while the vapor flow rate steadily increases as one proceeds up the stripping section. One of the problems arisen during the optimization of this process is the large amount of involved variables requiring a great number of simulation runs. Batista et al. (1998) employed

factorial design and surface response analysis for the optimization of the extractive SRV distillation process with ethylene glycol for the dehydration of ethanol obtaining a 57.5% energy saving related to conventional extractive distillation, and reducing the number of simulation runs. Columns thermally integrated by this procedure can be operated at lower reflux ratio reaching the same degree of purity and reducing the energetic expenditures.

## 8. Conclusions

The possibility of obtaining a renewable, available, safe and effective source of energy is one of the challenges that humanity should face. The biofuels, particularly the bioethanol, are an environmentally clean source of energy. However, production costs of fuel ethanol are higher than production costs of gasoline in some cases, although there is a strong influence of factors as the prices of oil and feedstocks for ethanol production. Nevertheless, many groups and research centers in different countries are continuously carrying out studies aimed at reducing ethanol production costs for a profitable industrial operation. Diverse research trends and process improvements could have success in the task of lowering ethanol costs. These research tendencies are related to the different steps of processing, nature of utilized feedstocks, and tools of process engineering, mainly process synthesis, integration and optimization. Process engineering could provide the means to develop economically viable and environmentally friendly technologies for the production of fuel ethanol. The most important and promising research priorities linked to process engineering for improving the global process are briefly summarized in Table 4.

An important part of the research trends on fuel ethanol production is oriented to the reduction of feedstock costs, especially through the utilization of less expensive lignocellulosic biomass. In general, most of the research efforts are oriented to the conversion of lignocellulosics into fermentable sugars and useful intermediates (due to the recalcitrance or resistance of the biomass to be converted). The key factor for enhancing the competitiveness of biomass-to-ethanol process is the increase in the specific activity of cellulases and the decrease in their production costs. In addition, the technology of recombinant DNA will provide important advances for the development of fuel ethanol industry. The development of genetically modified microorganisms capable of converting starch or biomass directly into ethanol and with a proven stability under industrial conditions will allow the implementation of the consolidated bioprocessing of the feedstocks.

Process synthesis will play a very important role in the evaluation of different technological proposals, especially those related to the integration of reaction–separation processes, which could have the major effects on the economy of the global process. Similarly, the integration of different chemical and biological processes for the complete utilization of the feedstocks should lead to the development of big "biorefineries" that allow the production of large amounts of fuel ethanol and many other valuable co-products at smaller volumes, improving the overall economical effectiveness of the conversion of a given raw material. Integration opportunities may provide the ways for a qualitative and quantitative improvement of the process so that not only techno-economical, but also environmental criteria can be met.

The increasing energy requirements of the humanity will augment the pressure over R&D centers, both public and private, for finding new renewable sources of energy and for optimizing their production and utilization. The employ of bioethanol as an energy source requires that the technology for its production from lignocellulosic biomass be fully developed at mid-term. This need is much more urgent for those countries that do not have the agro-ecological conditions for the cultivation of energy-rich crops like sugar cane, as the case of North American and European countries. Even from governmental biofuel programs in USA, the retrofitting of ethanol industry from corn starch to lignocellulosic residues (corn stover, woody materials, and municipal solid waste) has been recommended. Colombia presents a privileged situation in this field considering the great availability of the three types of analyzed feedstocks. Although the more logic option is sugar cane, social benefits for rural communities when other alternative feedstocks like cassava or typical agricultural and tropical residues should be taken into account.

Current development of ethanol industry shows that complex technical problems affecting the indicators of global process have not been properly solved. The growing cost of energy, the design of more intensive and compact processes, and the concern of the humanity by the environment, have forced the necessity of employing totally new approaches for the design and operation of bioethanol production processes, quite different to those utilized for the operation of the old distilleries. Every time, the spectrum of objectives and constraints that should be taken into account for the development of technologies for biofuels production is wider and more diverse. The social-economical component involved during the production of biofuels in the global context should be highlighted. Practically, every country can produce its own biofuel. In this way, the feedstock supply for ethanol production is "decentralized" and does not coincide with the supply centers of fossil fuels. This would make possible that the energetic dependence on oil of the countries intended to use biofuels at high scale could considerably decrease. In addition, human development indexes could be improved by two ways: creation of new rural jobs and reduction of gas emissions with greenhouse effect. However, ethanol production costs are higher than those of the fossil fuels, especially in the case of biomass ethanol. Nevertheless, during the last two years, oil prices have persistently increased. There is no doubt that the price of

Table 4
Research trends and priorities for improving fuel ethanol production by means of process engineering

| Issue | All feedstocks[a] | Sugar cane | Starchy materials | Lignocellulosic biomass |
|---|---|---|---|---|
| Process synthesis | Process synthesis by different approaches (e.g. optimization-based process synthesis) | Integration of ethanol production from sugar cane with facilities for ethanol production from cane bagasse | Integration of corn dry-milling plants with facilities for ethanol production from corn fiber | Process flowsheet development considering different pretreatment methods |
| Process analysis | Improvement of process control and operation (e.g. modeling, non-linear analysis)<br>Improvement of simulation and optimization tools (e.g. optimization under uncertainty, metabolic-flux models, etc.) | | | Full pilot-plant process analysis, especially for continuous processes |
| Process integration | Integration of fermentation and separation processes for reduction of product inhibition | Development of efficient co-generation technologies using cane bagasse | Development of CBP | Increase of effectiveness of SSF and SSCF processes (e.g. by improvm. of cellulase activity) |
| | Application of membrane technology (e.g. for ethanol removal or dehydration) | | Recombinant microorganisms for conversion of starch into ethanol | Development of CBP |
| | Energy integration (e.g. by pinch technology) | | | Increase of ethanol tolerance in microorganisms converting cellulose into ethanol<br>Development of recombinant microorganisms for CBP<br>Development of efficient co-generation technol. using solid residues of the process (as BIG/CC) |
| Other process engineering issues | Improvement of environmental performance considering the whole life cycle of bioethanol<br>Production of valuable co-products (retrofit to biorefineries)<br>Integrat. with petrochem. industry (e.g. ETBE prdn.) and biofuels production (e.g. biodiesel) | | | |

[a] Refers to the three analyzed groups of feedstock: sugar cane, starchy materials and lignocellulosic biomass.

gasoline and other oil-derived fuels has a subsidy paid by all taxpayers of the world and that is not necessarily made effective in gas stations. This "subsidy" is intended to compensate the inversions made for maintaining the *status quo* of international relationships. Logically, we also pay the consequences of the measures taken to "offset" this state of things: social instability and, unfortunately and in a certain degree, terrorism.

Therefore, the relatively higher production cost of ethanol is the main obstacle to be overcome. To undertake this, process engineering plays a central role for the generation, design, analysis and implementation of technologies improving the indexes of global process, or for the retrofitting of employed bioprocesses. Undoubtedly, process intensification through integration of different phenomena and unit operations as well as the implementation of consolidated bioprocessing of different feedstocks into ethanol (that requires the development of tailored recombinant microorganisms), will offer the most significant outcomes during the search of the efficiency in fuel ethanol production. Great efforts should be focused in the development of CBP of biomass as lignocellulosics is the most promising feedstock for ethanol production. Additionally, the intensification of biological processes implies a better utilization of the feedstocks and the reduction of process effluents improving the environmental performance of the proposed configurations. Attaining this set of goals is a colossal challenge to be faced through the fruitful interaction between the biotechnology and the chemical engineering.

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