Biofuel Production Technology and Engineering

Hugo Iván Velázquez-Sánchez, Alexis Saldivar-García and Ricardo Aguilar-López

Abstract Biofuels are leading a group of alternative energy sources due the fact they can make use of organic waste as feedstock and be more environmentally friendly than fossil ones. One of the most attractive ones is the use of butanol as a gasoline enhancer or substitute, as both compounds share significant physico-chemical properties such as energy content. Current research results show that it's possible to use agro-industrial waste as feedstock thanks to the discovery of new species and saccharification technologies. In this work a basic outline of the state-of-the-art overview of biofuel technologies, their properties and current challenges is presented. The potential of the use of saccharification processes into biofuel producing ones as a way to take advantage of the wide array of agro-industrial waste currently generated as feedstock is discussed, and finally a brief introduction to the ABE (acetone-butanol-ethanol) fermentation system is given, as it is a pathway for butanol production by biological means by the bacterium *Clostridium*. Although wide array of sugars can be used, some of the current challenges and strategies to address the problems inherent to the biological system, such as low productivities and inhibitory effects caused by solvents accumulation into the reactor is discussed. Finally, this chapter will close with a brief analysis of the scope of these strategies within the context of bioprocess engineering, showcasing the efforts made in this context to adapt new fermentation regimes to increase the system's butanol productivity based on modelling and simulation techniques.

Keywords Agro-industrial waste · Lignocellulose · Saccharification · Biofuels · Butanol · *Clostridium*

H.I. Velázquez-Sánchez · A. Saldivar-García · R. Aguilar-López (✉)
Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional,
Av. Instituto Politécnico Nacional No. 2508, San Pedro Zacatenco,
07340 Mexico City, Mexico
e-mail: raguilar@cinvestav.mx

O.P. Karthikeyan et al. (eds.), *Recycling of Solid Waste for Biofuels and Bio-chemicals*, Environmental Footprints and Eco-design of Products and Processes, DOI 10.1007/978-981-10-0150-5_10

1 Introduction

It is well known that humanity is currently experiencing a major energy crisis because the main source of it comes from a non-renewable resource such as oil. The reality of the oil sector indicates that reserves are at a critical level, therefore now research effort is focused on the study and development of processes aimed to consolidate alternative energy production and distribution technologies (Demirbas 2009).

One of the key objectives set out in the approach to the use of alternative energy technologies is to reduce the environmental impact caused by both the exploitation and use of fossil fuel energy, especially considering the great environmental harm caused by extraction, refining and use of oil derivatives, which represents a threat to both terrestrial and marine ecosystems due to leakage of aromatic hydrocarbons (Brown 2003). Although organisms of bacterial or plant origin can remediate contaminated sites, is also known that many of these organisms may produce intermediate compounds that could exhibit greater toxic effect on the biota of affected sites than the original contaminants. Additionally, the use of petroleum fuels in conventional internal combustion engines also generates toxic compounds, resulting from the partial oxidation of these and their impurities, that appreciably contribute to increase the emission of greenhouse effect gases as CO_2, CH_4, NOx and SOx (Escobar et al. 2008).

Therefore, when looking for strategies to develop and exploit alternative energy sources, greater emphasis on generating strategies to provide added value to the operation should be given, as this aims to increase the success rate of the process for scale-up to commercial levels without suffering undue pressure from an economic perspective (Dufey 2006).

Within the overall progress made in research and development of alternative energy technologies, a vast set of technologies exists aimed to harness sunlight, air currents, geothermal activity or nuclear energy. However, although there is a wide range of possibilities, most of the technologies currently developed for the exploitation of these sources have very low conversion yields and high infrastructure costs associated with their exploitation. In addition, it must be considered that these types of alternative energy sources are often limited to generate electricity, but cannot easily cover the growing problem of fuel supply for the transport sector (Melaina et al. 2013). Attempts to incorporate the above mentioned technologies not only requires implementation, for example, of a solar panel system, but also the modification of the engine and its mechanical system to make it compatible with this new technology, and that would arise the need to replace almost all of existing vehicles today by those that could function with the new power supply, which implies further economic and social issues (Ogden et al. 2004).

It is noteworthy that none of the technologies presented in the previous paragraph meet the criteria mentioned above of adding additional benefits outside reducing pollution derived from their use, especially considering that another concerning issue today is the current rate of waste generation and the environmental pollution caused by the various human activities, and the use of renewable energies like wind and solar ones do not provide direct answers to said problems.

Biofuels, which can be defined as all compounds of organic nature derived from living beings and their metabolism, lead a group of alternative energy sources aimed to provide solution to the issues raised above, as they seem more suitable to address specific issues that other technologies cannot overcome, such the use of organic waste as raw material (Maddipati et al. 2011). Additionally, biofuels processes have extensive theoretical knowledge that supports them, as fermentation technology is almost as ancient as humanity (Naik et al. 2010). However, current research and development of biofuel technologies cannot achieve reasonable production levels to make them attractive from the economic point of view due both low conversion yields and availability of feedstock materials.

One of the most studied biofuels in the last decade is butanol, as opposed to the currently developed processes for the production of ethanol and biodiesel, which could offer better answers regarding fuel mileage yield, lower gaseous emissions and better physico-chemical properties while also being able to make use of agro-industrial waste as feedstock requiring minimal or null pre-treatment to obtain it (Lee et al. 2008a, b).

Therefore in this chapter there will be a general showcase of the state-of-the-art biofuel technology, which leads to the current advances and challenges of said technologies regarding the use of agro-industrial waste as feedstock, followed by a brief discussion about the down-side of the use of ethanol as fuel and the advantages offered by butanol for the automotive sector. Then butanol production via bioprocesses and the advances made in this field of bioengineering to make use of biomass-derived residues as substrate will be discussed. Finally, efforts made in our work group to find novel fermentation techniques that could allow to improve the productivity of the butanol producing systems from a kinetic modelling and simulation perspective will be introduced.

2 Biofuels

Biofuels can be classified according to the aggregation state in which they are useful as fuels. Within gaseous biofuels, the best known representative is the so-called biogas, which is a mixture consisting primarily of methane, carbon dioxide and other trace elements. This type of fuel is also found relatively easily in the environment and can come from natural sources, such as swamps or lakes, or from anthropogenic sources such as livestock. The main virtue of this type of

compounds is their high calorific value, whereby large volumes of gas are not required to achieve acceptable production of energy. However the most important issues to overcome before its use as a reliable energy source is the large amount of infrastructure needed to efficiently collect such compounds and the difficulty and risk for its transport and storage, since pressure and temperature of the vessels and ducts employed for such task need a strict control to prevent explosions.

Solid biofuels, made up mostly of plant biomass as wood, straw or coal are abundant in nature. Yet, uncontrolled combustion does result in the generation of ashes with high contents of heavy metals or gaseous emissions containing sulphur or nitrogen oxides that not only contribute to increasing the greenhouse effect, but strong acids in the presence of atmospheric water vapour can be formed which precipitate as acid rain (Vamvuka et al. 2003). In addition, the process in which they can be obtained, can further affect the ecological balance by requiring the destruction of large-scale forest ecosystems.

Finally, for liquid biofuels, a wide array of compounds exist but, unlike solid or gaseous biofuels, liquid ones are not easily found in the environment. These are generally obtained mostly through fermentation processes performed by microorganisms of bacterial or fungal origin, or generated by algae with high lipid content (Gomez et al. 2008). Within this scenario, it cannot be anticipated that liquid biofuels would share most of the problems of exploitation, management or distribution of solid and gaseous ones, since most of the internal combustion systems are based on the use of liquid fuels; however these compounds still suffer from low conversion yields and, in some cases, high recovery costs of finished products. In contrast to the classic fossil fuel's production processes, such renewable biofuels tend to be restricted to recoverable or profitable compounds of interest, which affects the economic viability of their production (Pfromm et al. 2010).

Another current issue with biofuel technology is feedstock availability and price, as traditional biotechnological processes require the use of monomeric or dimeric sugars as substrate for cell growth (McNew and Griffith 2005). Nonetheless, it is known that within the diversity of waste generated due world trade and industrial activities, there is a vast variety of hydrocarbonated components that can be utilized as raw material for producing biofuels (Sharma et al. 2013).

3 Agro-Industrial Waste

In this context, agro-industrial waste can be defined as those residuals of organic nature generated or derived from the use, collection and processing of plant biomass. The agro-industrial wastes consist of a variety of components that can come from various sources. There are those generated by the exploitation of forest resources, such as tree bark, wood chips, sawdust, remains of chipboard, etc. that are not used efficiently and are not integrated into finished products or from

agronomic nature which include stubble remains of fruits and vegetables, straw, seed shells, waste from processes for obtaining syrups or juices, pulp, etc. (Singh et al. 2012). These compounds mainly contain sugars arranged into molecules of high molecular weight that exhibit significant amounts of radical branching, cyclization and physico-chemical properties that confer biochemical stability, rendering them unsuitable for traditional fermentation (Lo et al. 2008).

Therefore, based on the composition and structure of the various agro-industrial residuals, a classification can be made in advance to propose an adequate mechanism for their decomposition into fermentable sugars and subsequent transformation into fuels.

Waste from logging and conversion of forest resources consist mainly of what is called lignocellulose, which is composed of cellulose fibres wrapped in an amorphous matrix of hemicellulose chains and skeletons of lignin (Martinez et al. 2009). It has a high mechanical and chemical resistance due to cyclic aromatic groups present in the matrix of lignin, rendering them more or less resistance against attack by microorganisms and preventing direct use as raw materials for liquid biofuels despite high availability (Yuea et al. 2014).

Cellulose is the most abundant organic polymer in nature. It is made up of long chains of glucose, usually linked by covalent bonds β-1,4. Glucose polymers forming the cellulose have a linear structure and tend to bind together by hydrogen bridge links, generating what is known as crystalline region, where cellulose fibres are organized into compact structures which is the reason behind their high chemical stability and resistance to attack by biological agents (Klemn et al. 2005). Additionally, there is another region within cellulose chains termed amorphous region, conformed by glucose polymers that do not arrange so tightly due a reduced formation of hydrogen bonds between the chains; thus amorphous regions are more susceptible to chemical and biological degradation (Aro et al. 2005).

In contrast, hemicellulose is a heterogeneous polymer, consisting of monomers bearing no specific type of sugar or with a single type of bond between them, making it more likely to have no crystal structures (Aspinall 1959). This polymer is comprised mostly of pentose sugars such as xylose or arabinose, hexoses such as mannose, glucose or galactose and uronic acids (Gírio et al. 2010). These chains tend to link to the cellulose through hydrogen bonds or by interaction with other polymers such as lignin and pectin. Hemicelluloses have a relatively short chain length with respect to cellulose, however this kind of polymer can represent from 15 to 35 % of the total dry weight of the plant material (Scheller and Ulvskov 2010).

With the statements in the preceding paragraphs it is easy to understand the importance of generate technologies that allow the use of the large amount of organic matter present in agro-industrial waste, not only to solve problems related to environmental pollution but also to take advantage more efficiently of natural resources and guarantee the supply of raw material for renewable liquid biofuel production to ensure economic and technical feasibility.

4 Saccharification Methods

Obtaining sugars from the structural constituents of plant biomass is not a novel research topic, as since the composition of the polymers described in the previous section are known, sugar extraction attempts for commercial purposes, particularly within the area of food supply, have been extensively studied (Harris 1949). The biggest obstacle present is inherent to the physico-chemical nature of agro-industrial waste which has hampered incorporating them and the use of chemical or physical agents that jeopardize both the product integrity, quality and safety for both human and bacterial feedstock (Sun and Cheng 2002).

The first techniques of degradation of plant biomass were based on the process developed during World War II by Giordani in 1939 (Kobayashi et al. 1962). These techniques involved the use of size reduction operations, such as grinding or milling, the resulting chips is given a chemical treatment with dilute sulphuric acid to remove or eliminate the hemicellulose present in the plant tissue, then the biomass is subjected to a treatment with a concentrated acid solution containing up to 60 % H_2SO_4 and allowed to dry to be then re-treated with the liquor obtained from the first stage of hydrolysis in a container at high temperature. This process has the advantage of having very good recovery yields of sugars, which provides concentrated sugar solutions for fermentation, however high consumption of acid solutions and the presence of inhibitory compounds for bacterial growth (such as furfural) require additional treatment for the use as feedstock (Qureshi et al. 2007).

Another hydrolysis techniques also involves the use of acid but exclusively in diluted form. Here the biomass is crushed and then treated with sulphur dioxide gas, the chips are then heated to 180 °C for 2–3 min and then compressed by expansion valves to generate a pulp. This pulp is subsequently washed and the soluble components recovered into the supernatant is subjected to a new round of contact with dilute sulphuric acid. This technique allows to obtain fermentable sugar solutions with up to 8 % w/w and without high concentrations of inhibitory compounds that could affect the bacterial growth for carrying out fermentation (Saha et al. 2005).

Finally, another chemical treatment performed to obtain fermentable sugars from plant biomass is the use of alkaline solutions, which may contain as active compound sodium or ammonium hydroxide. This treatment is, however, only used during pre-treatment stages, as they help to generate porosity into the cellulosic material and thereby allows for increased contact surfaces between the solid phase and the liquid medium containing hydrolysis agents, such as enzyme preparations (Chen et al. 2013a, b).

The techniques of biological degradation of lignocellulosic material is a more recent research topic, which has emerged due to the large amount of knowledge generated in the areas of bioengineering and molecular biology that allows a more detailed study of the organisms that have the capacity of feeding on such complex substrates or agro-industrial waste (Demirbas 2009). These technologies promise higher performance of polymer conversion to fermentable sugars due to use of

highly specific enzymes involved in the hydrolysis. An additional advantage is the requirement of less aggressive operating conditions such as pressure, temperature and pH compared to chemical methods (Sandgren and Hiberg 2005). Despite this, the cost of producing the enzymes required to perform the operation is relatively high and the organisms, that produce proteins with such properties naturally, have complex nutritional requirements and require facilities that allow for solid state fermentation to optimize production of these enzymes (Pandey 2003).

5 Current Challenges of Biofuel Production

One of the processes currently established at large-scale is ethanol production as an additive or substitute for gasoline in internal combustion engines, which, despite being one of the oldest biotechnology techniques and with extensive knowledge derived from that vast experience, still requires additional efforts to become a solid long-term candidate to replace fossil fuels (Lee et al. 2008a, b). First of all it must be mentioned that the type of raw material (sugar cane and corn, respectively) used for production in Brazil and in the United States, which are the largest producers of ethanol for use as biofuel, comes from resources originally destined for both animal and human consumption, (Pimentel et al. 2007). Added to this, ethanol has technical limitations of use, storage and integration into current internal combustion engine technology, such as being more hygroscopic with respect to oil-based fuels. Therefore it cannot be transported efficiently over long distances through pipes without accumulating additional moisture, thus reducing its final titter, and contributing to internal oxidation of the pipes (Antoni and Zverlov 2007).

With the above, different biofuels have been looked for in nature to attempt to lessen the issues previously stated, and one of the most convincing candidates is butanol.

6 Butanol as Biofuel

Butanol is a short chain alcohol, which has been produced by biological means for a long time. It has the advantage of a higher energy content per litre of fuel than ethanol, less volatility and slightly lower octane number than gasoline, which can improve its yield over the ethanol and reduce engine gaseous emissions. Also due to higher density, a higher mass of fuel is injected into the engine, which, considering its similar calorific power versus gasoline, helps to attain comparable energy content per litre. It is therefore assumed that butanol would be a better fuel extender or substitute for current regular gasoline than ethanol (Table 1).

Traditionally, the methodology for obtaining butanol by fermentation is based on the degradation of various sugars (particularly glucose or sucrose) carried out by Gram-positive bacteria of the genus *Clostridium*, via a metabolic pathway called

Table 1 Comparison of different physico-chemical properties between regular gasoline, ethanol and n-butanol (Modified from Mužíková et al. 2014)

Fuel	Density (kg/m^3)	Reid vapour pressure (kPa)	Energy content per litre (MJ/L)	Research octane number	Solubility in water @ 20 °C (w/w)
Regular gasoline (US/UK)	740	45–90	32.6	95	0.01
n-butanol	810	2.3	29.2	94	20.1
Ethanol	794	17	23.5	106	Completely miscible

ABE (acetone-butanol-ethanol) (Qureshi et al. 2008). This biological process was originally exploited for the production of acetone as a solvent for the chemical and military industry, being of such importance that most of the explosives made and used during World War II were based on this technology. Back then, butanol was considered an undesirable product, however, the former Soviet Union began its use as fuel, partly due to the harsh weather conditions. By the mid-60s the process of obtaining ABE products at industrial-scale allowed to reach reaction volumes up to 300 m^3. Nonetheless, the boom in the petrochemical industry in the 70s provided solvents and chemicals at very low cost compared to the biological process, resulting in fermentation plants being dismantled (Zverlov et al. 2006).

Currently, there is a renewed interest in the study of the ABE system in order to adapt such technology to suit the energy demands of this time (Lee et al. 2008a, b). Still, biological system present native restrictions that have prevented consolidation as a mature technology such as low production yields, inhibitory effects of accumulated solvents with culture age and mechanisms of metabolic regulation present in Gram-positive bacilli, such as sporulation (Zheng et al. 2009).

7 *Clostridium* Cultures Using Agro-Industrial Waste as Substrate

One of the biggest problems of traditional fermentation processes, which are generally based on yeast cultures for the production of alcohol, is that the organisms do not have the ability to degrade and consume pentoses constitutively. This is a desirable attribute in the context of use of agro-industrial waste as feedstock for the generation of biofuels, because of the large amount of hemicellulose present in the structure of residues coming from both the wood and paper industry (Martín et al. 2007). Studies in the past decade have been conducted to find new species, use molecular techniques or metabolic engineering in order to generate strains with the ability to growth on a wider range of carbon sources. Generated strains existing today exhibit moderate yields of alcohol production which do not surpass titters

over 20 g L^{-1}, preventing consideration as strong candidates for biofuel production (Atsumi et al. 2008; Peralta-Yahya et al. 2012).

In contrast, many strains of *Clostridia* display natural capabilities to metabolise a wider range of carbon sources including pentoses, being most attractive the species that can feed on xylose and mannose, which are the most abundant monosaccharides in w/w fraction generated from saccharification of lignocellulosic residues (Qureshi et al. 2007).

In addition, it is known that some wild-type *Clostridium* species have the ability to grow solely on lignocellulosic carbon sources, such as *Clostridium thermocellum* or *Clostridium celullolyticum* (Bayer et al. 1983; Keis et al. 2001). These species are widely recognized within the processes of degradation of plant biomass because they possess the ability to produce extracellular cellulolytic enzymes in large quantities organized into complex structures called cellulosomes (Lamed et al. 1983a, b). Within a cellulosome, a wide array of endoglucanases, cellobiohydrolases, xylanases and other degradative enzymes exist that allow the microorganisms to efficiently degrade almost all of the cellulosic or hemicellulosic substrate found in vegetal biomass (Nordon et al. 2009).

Table 2 summarizes current advances in ABE fermentation using agro-industrial waste as feedstock and the highest producing strains. It is noteworthy to emphasize that there are technologies that can reach practical yields that match the theoretical value of 0.45 g/g of ABEs from glucose, which is indicative that the research in this field is stepping in the right direction.

Table 2 Summary of the results of ABE production under batch fermentation (Modified from Liu et al. 2013)

Feedstock	Strain	ABE concentration (g/L)	ABE yield (g/g)	ABE Productivity (g/L * h)	References
Barley straw	*C. beijerinckii*	26.64	0.43	0.39	Qureshi et al. (2010)
Wheat straw	*C. beijerinckii*	21.42	0.41	0.31	Qureshi et al. (2008)
Corn fiber	*C. beijerinckii*	9.3	0.39	0.10	Qureshi et al. (2008)
Corn stover	*C. beijerinckii*	26.27	0.44	0.31	Qureshi et al. (2010)
Rice straw	*C. saccharoperbutylacetonicum*	13	0.28	0.15	Soni et al. (1982)
Bagasse	*C. saccharoperbutylacetonicum*	18.1	0.33	0.3	Soni et al. (1982)
Switch grass	*C. beijerinckii*	14.61	0.39	0.17	Qureshi et al. (2010)

(continued)

Table 2 (continued)

Feedstock	Strain	ABE concentration (g/L)	ABE yield (g/g)	ABE Productivity (g/L * h)	References
Domestic organic waste	*C. acetobutylicum*	9.3	0.38	0.08	Claassen et al. (2000)
Sago	*C. saccharobutylicum*	16.38	0.33	0.59	Liew et al. (2005)
Defibrated sweet potato slurry	*C. acetobutylicum*	5.87	0.29	0.12	Badr et al. (2001)
Cassava	Co-culture of *B. subtillis* and *C. butylicum*	9.71	0.21	0.135	Tran et al. (2010)
Crystaline cellulose	Co-culture of *C. thermocellum* and *C. saccharoperbutylacetonicum*	10.3	0.25	0.02	Nakayama et al. (2011)
Deshelled corn cobs	Co-culture of *C. cellulovorans* and *C. beijerinckii*	11.8	0.17	0.14	Wen et al. (2014)

8 Current Challenges for the ABE Fermentation Processes

Although, as described above, it can be assumed that the problem of availability of substrate for butanol production as a biofuel could be guaranteed, it should be mentioned that generally, most of the obstacles that impede the use of clostridial strains in ABE producing bioprocesses involve the biochemical limitations to bacterial growth by both substrate and solvents concentrations in the broth. Some authors (Lee et al. 2008a, b; Qureshi et al. 2010) report that ABE producing bacteria will not grow in solutions containing over 175 g/L of glucose or 14–21 g/L of solvents, as excess carbon sources disrupt incorporation by the microorganisms and the high solvent titters partially solubilize the cell's wall causing a depolarization of their membranes, which impedes stabilization after or during cell division.

Additionally, the production of butanol using agro-industrial waste as feedstock, needs to consider pre-treatment and saccharification techniques, as both acid and alkaline methods have a negative impact on growth of the culture because clostridial species regulate their metabolism. They sense hydrogen potential gradients between their cytoplasm and their surroundings, resulting in acid media causing what is it known as acid crash and alkaline ones cause inhibition due to poor proton exchange (Dürre 2007). Finally it should be noted that *Clostridium* species with cellulolytic capabilities usually do not have the enzymes necessary to perform ABE fermentation, so these strains cannot be used by themselves in the production processes of second generation biofuels (López-Contreras 2001).

Research advances made up to date utilise both molecular biology and engineering techniques to solve some of the issues of renewable butanol for biofuel

production from agro-industry wastes. Genetic manipulation target overexpression of the key transcription factor that regulates sporulation (*spoA*) coupled to the downregulation of the expression of hydrogenases encoded by the gene cluster known as *hupCBA*. This approach resulted in increased solvent production in batch culture and reduction of inhibitory effects caused by the accumulation of solvents in the medium (Alsaker et al. 2004). Additionally there are reports of metabolic engineering via antisense RNA, targeting the transcript of the gene *ctfB*, to redirect the carbon flux from acetone forming pathways to butanol producing ones, has had moderate success (Tummala et al. 2003).

Recent studies using an engineering approach have explored the feasibility of implementing strategies for unconventional culture techniques in which it is proposed to feed a first reactor directly with agricultural shredded waste and inoculate it with bacteria of the genus *Clostridium thermocellum* to obtain fermentable sugars that could feed to a second reactor inoculated with *Clostridium acetobutyllicum* or *Clostridium beijerinckii* to obtain ABE products (Qureshi et al. 2007). These studies have shown that success can be further enhanced by implementing a co-culture of both a cellulolytic and a butanol-producing strain in the same reactor (Nakayama et al. 2011).

Despite all advances made in the ABE technology, these have been unable to achieve optimal yields based on capabilities of the *Clostridium* strains. Particularly, as yields are typically based on empirical knowledge, process efficiency cannot be assessed with regards to process engineering, such as analysis, design, optimization and process control.

Formulation of mathematical models could overcome such challenges if they could describe and, ideally, also predict the behaviour of the biological system under study over a wide range of operational conditions. This would allow to determine more appropriate procedures for the exploitation of the bacterial metabolism more precisely. In this light, it is necessary to analyse the ABE pathway to determine best strategies to successfully achieve the intended goal.

9 ABE Metabolic Pathway

The ABE metabolic pathways is composed of 19 main reactions (Fig. 1), in which acetate, butyrate, ethanol, acetone, lactate and hydrogen are co-products of the fermentation. The metabolism shows two distinctive phases, an acidogenic phase, in which acetate and butyrate are the main products during exponential growth phase of the culture, and a solventogenic phase, in which butanol, ethanol and acetone are the main products relating to stationary phase (Bahl et al. 1982).

Hexoses are metabolised into pyruvate via the Embden-Meyerhof-Parnas pathway, whereas pentoses are incorporated into the metabolism by the action of the UDP-glucose uridyl transferase (Durán-Padilla et al. 2014). Pyruvate is one of the key intermediates of the *Clostridium* metabolism, and under certain conditions *Clostridium* species are capable of transforming pyruvate to lactate through

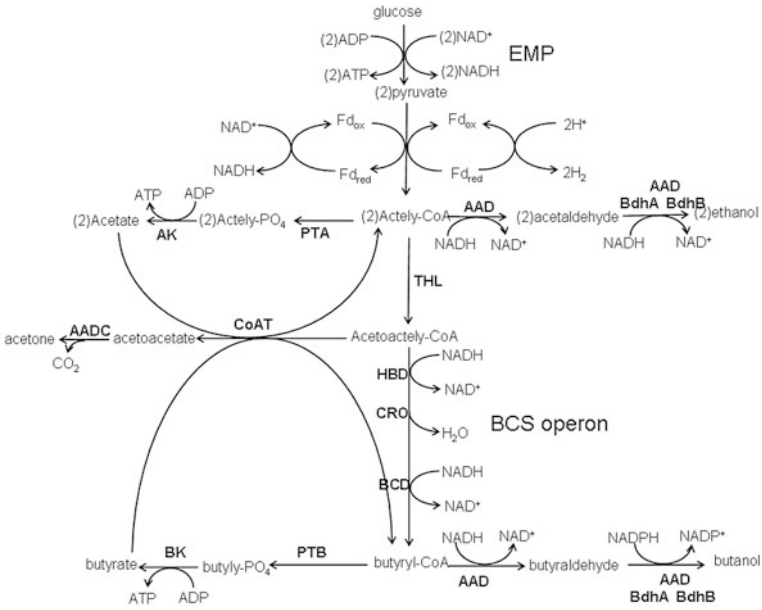

Fig. 1 ABE metabolic pathway

pyruvate dehydrogenase. The main reaction, however, is conversion of pyruvate to acetyl-CoA through pyruvate-ferredoxin oxidoreductase (Uyeda and Rabinowitz 1971) with the concurrent reduction of ferredoxin, a reason why lactate formation is not considered in most metabolic models.

NAD(P)H-Ferredoxin oxidoreductases are key enzymes for electron transport in the *Clostridium* genus (Gheshlaghi et al. 2009). In acid-producing cultures, ferredoxin-reductase shows high activity. Two reasons have been proposed, (i) necessity for regenerating NAD^- consumed in the Glyceraldehide-3-P-dehydrogenase reaction, or (ii) as acidogenic cultures are in exponential growth, high enzymatic activity is required for meeting energy requirements. The ferredoxin oxidoreductases regulatory mechanism appears to be related to NAD- and NADH concentration, which turns out to be an efficient regulatory system that prevents accumulation of NADH in the acidogenic phase, and increasing $NAD(P)^-$ reductases activity during the solventogenic phase in turn, for the higher NAD(P)H demand needed for ethanol and butanol production in that stage (Gheshlaghi et al. 2009).

Hydrogen formation is catalysed by the hydrogenase, which uses reduced ferredoxin as electron donor (Gheshlaghi et al. 2009). As well as ferredoxin reductases, the hydrogenase shows distinctive behaviour during acidogenesis and solventogenesis, as reduced ferredoxin competes with $NAD(P)^-$ reductases for it, meaning it also competes for reducing power with butanol and ethanol dehydrogenases during solventogenesis.

The ABE pathway has three important nodes in Acetyl-CoA, Acetoacetyl-CoA and Butyryl-CoA. Acetyl-CoA is a rigid node from which the carbon flux is

distributed to five different metabolites. Two branches come out directly from Acetyl-CoA which end up in acetate and ethanol, from Acetoacetyl-CoA acetone is produced while butanol and butyrate are produced from butyryl-CoA. Acetate and butyrate are energy producing reactions in which 1 molecule of ATP is produced for each Acetyl/Butyryl-CoA consumed. Both compounds are re-assimilated during solventogenesis, this reaction is catalysed in two different ways, acetate and butyrate can either be converted to acetyl/butyryl-CoA through a reversible reactions or through the action of the CoA-transferase (Millat et al. 2014), which consumes one molecule of Acetoacetyl-CoA and one of acetate/butyrate to produce one molecule of acetoacetate and one of acetyl/butyryl-CoA. This mechanism sets up an efficient solvent producing strategy, given that it would be energetically unfavourable to re-assimilate both acetate and butyrate through the reversible reactions, the availability of a non-ATP consuming reaction lets the organism face the energy deficit while still being capable to produce ethanol and butanol in the stationary or solventogenic phase.

This theory is supported by findings made by Desai et al. (1999) and Lehmann et al. (2012) indicating that butyrate consumption during solventogenesis is catalysed through the reversible reaction, as opposed to acetate consumption which is related to acetoacetate production. Also Wang et al. (2013) and Desai et al. (1999) reported that acetate is produced even after the organism shifted to solventogenesis, which would be an indication that the production of acetate is necessary for energy generation.

There are several hypothesis trying to explain the factors that control the switch between acid generating metabolism and solvent generating metabolism. Some authors (Hüsemann and Papoutsakis 1988; Terracciano and Kashket 1986) suggest that the concentration of un-dissociated butyric acid is the controlling factor. However, Chen and Blaschek (1999) proposed that the switch is the result from extracellular and intracellular signals, like culture's pH or intracellular Acetyl-P and Butyryl-P concentrations respectively. Zhao and Tomas (2005) concluded that it is Butyryl-P and not Acetyl-P that controls the metabolism switch, while Wietzke and Bahl (2012) suggest that energy and redox balance are the key intracellular signals to start solvent production.

10 ABE Fermentation Kinetic Modelling

According to Mayank et al. (2012) and the authors knowledge there are 7 relevant kinetic models for ABE fermentation reported in literature (Shinto et al. 2007; Li et al. 2011a, b; Haus et al. 2011; Napoli et al. 2011; Thorn et al. 2013; Millat et al. 2014; Velázquez-Sánchez et al. 2014), moreover Metabolic Flux Analysis has been used to successfully describe the behaviour of the ABE pathway (Desai et al. 1999; Papoutsakis and Rice 1984). However, for the sake of simplicity and reproducibility, all reported kinetic models have been validated through experiments conducted using glucose as the only carbon source. Therefore, to

ensure a fair comparison between results of this and the following sections of the chapter, all selected analyses are based on studies made under that condition, which implies that the fermentation stage of the analysed operational regimes are made after an upstream operation of lignocellulosic digestion.

Five of the six proposed models are structured metabolic models, which intend to recreate the bacterial metabolism at both an enzymatic and genetic level, but their inherent complexity makes it difficult to successfully apply them to the bioengineering field, such as in process design and optimization. Therefore the necessity for a model aiming to study the process at a much simpler level is evident. The model should hold sufficient predicting capacity to provide insight into the Clostridial metabolism and also help in the design of industrial-scale ABE fermentation.

Velázquez-Sánchez et al. (2014) set up the precedent of an unstructured phenomenological model that could describe the process with sufficient accuracy and in this revision an improved version of the model is used to study different working regimes and butanol production strategies. The kinetic model has a set of seven differential equations describing the behaviour of each product of interest. There are 6 production rate equations, one for each of the following compounds: Butanol (But), Acetone (Ace), Ethanol (Et), Acetate (Act), Butyrate (Sb) and Biomass (X). The biomass production model (Eq. 1) is a Haldane-Luong equation, taking into account the inhibitory effect of butanol, but not the one caused by the carbon source. Butyrate kinetics (Eq. 13) include a product formation rate (Eq. 5) considering glucose as its main substrate, and one consumption rate described by Eq. 2 for Butanol production.

$$\mu_x = mumaxx * \left(\frac{Sg}{ksg + Sg}\right) * \left(1 - \left(\frac{But}{kbut}\right)^m\right) \tag{1}$$

$$\mu_{but} = mumaxbut * \left(\frac{Sg}{ksgb + Sg}\right) * \left(\frac{Sb}{ksb + Sb}\right) \tag{2}$$

$$\mu_{act} = mumaxact * \left(\frac{Sg}{ksgact + Act}\right) \tag{3}$$

$$\mu_{et} = mumaxet * \left(\frac{Sg}{ksget + Sg}\right) \tag{4}$$

$$\mu_{sb} = mumaxsb * \left(\frac{Sg}{ksbsg + Sg}\right) \tag{5}$$

$$\mu_{ace} = mumaxace * \left(\frac{Sb}{kba + Sb}\right) * \left(\frac{Act}{kaa + Act}\right) \tag{6}$$

Butyrate consumption (Eq. 13) takes into account the reversible pathway that transforms butyrate into butyryl-P and, subsequently, into Butyryl-CoA, as well as the reaction catalysed by CoA-transferase. For acetate consumption, given the

results reported by Desai et al. (1999) and Lehmann et al. (2012), only the reaction catalysed by the CoA-transferase is considered.

$$\frac{dSg}{dt} = (D * (Sga - Sg)) - X * \left(\left(\frac{\mu_x}{Yxsg}\right) + \left(\frac{\mu_{but} * (Ybx)}{Ybutsg}\right) + \left(\frac{\mu_{act} * (Yactx)}{Yactsg}\right) + \left(\frac{\mu_{et} * (Yetx)}{Yetsg}\right) + \left(\frac{\mu_{sb} * (Ysbx)}{Ysbsg}\right)\right) \tag{7}$$

$$\frac{dX}{dt} = (-D * X) + (\mu_x - kd) * X \tag{8}$$

$$\frac{dAce}{dt} = (-D * Ace) + (\mu_{ace} * (X * Yacex)) \tag{9}$$

$$\frac{dBut}{dt} = (-D * But) + (\mu_{but} * (X * Ybx)) \tag{10}$$

$$\frac{dAct}{dt} = (-D * Act) + X * \left((\mu_{act} * (Yactx)) - \left(\frac{\mu_{ace} * (Yacex)}{Yaceact}\right)\right) \tag{11}$$

$$\frac{dEt}{dt} = (-D * Etoh) + (\mu_{et} * (X * Yetx)) \tag{12}$$

$$\frac{dSb}{dt} = (D * (Sba - Sb)) + X * \left((\mu_{sb} * (Ysbx)) - \left(\frac{\mu_{but} * (Ybx)}{Ybutsb}\right) - \left(\frac{\mu_{ace} * (Yacex)}{Yacesb}\right)\right) \tag{13}$$

Having declared the mass balance equations, it is necessary to analyse the behaviour of the biological system and its response under multiple operating conditions to ensure stability and reproducibility of the results, with a view to scaling and control system strategies, using this kind of novel modelling technique.

11 Butanol Production Strategies and Analysis

Little has been reported in the literature regarding the effect of the operating regime in establishing ABE fermentation using *Clostridium* strains and the possible causes and effects associated with butanol production due the use of one or another production strategy, as there are generally are limited studies available to evaluate a particular condition against classic batch methods, so many of the novel proposed processes are still open for analysis.

Therefore, the following four different production strategies are being analysed:

1. Adding acetate/butyrate at the beginning of a batch fermentation and in the inlet of a Continuous Stirred Tank Reactor (CSTR).
2. CSTR in one, two and three stages.
3. Fed-Batch culture.
4. CSTR followed by a fed-batch stage.

11.1 Batch Fermentation

It has been proved that adding butyrate at the beginning of a batch fermentation improves butanol yields and productivity (Chen and Blaschek 1999; Lee et al. 2008a, b; Chang 2010; Wang et al. 2013), due to a larger carbon pool shifts carbon flux toward butanol and because the presence of butyrate causes feed-back inhibition of its forming pathway (Lee et al. 2008a, b; Wang et al. 2013).

Figure 2 showcases the possible behaviour of the system feeding either butyrate or acetate at the beginning of fermentation. Simulations performed with 4 g L^{-1} of butyrate added at the beginning of fermentation shows that final butanol concentration after 300 h is 12.7 g L^{-1}, an improvement of 33.7 % from the 9.6 g L^{-1} of butanol achieved without butyrate. Analysing the product formation rate it becomes evident that although butanol maximum productivity does not exceed that of the one obtained by the culture without butyrate, butanol production begins earlier in the fermentation and continues over a longer period, which causes the final titter to increase. Acetone experiences the opposite effect, final concentration drops by 15 % caused by a reduction in its formation rate during solventogenesis. There is no

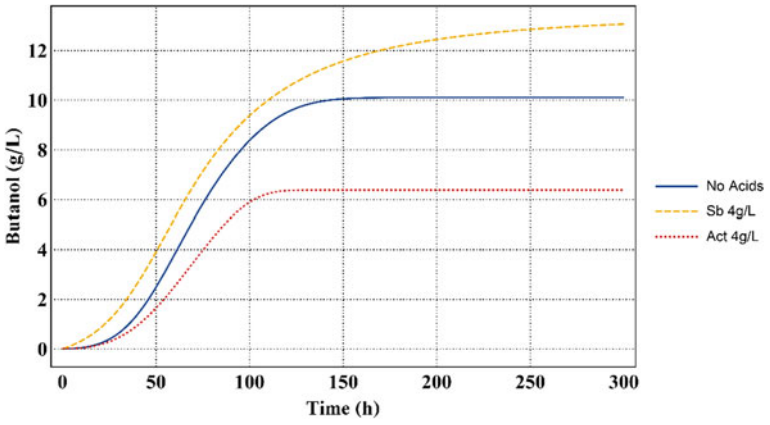

Fig. 2 Comparison of butanol production between classical batch fermentation vs. systems supplemented with butyrate or acetate at t_0 made by simulation using the author's kinetic model

agreement on the effect of adding acetate into a batch ABE fermentation. Chen and Blaschek (1999) report that the presence of acetate indeed increases both acetone and butanol final titters, however Holt et al. (1984) report that even though acetone concentration increases, butanol productivity experiences as much as a two fold decrease. Simulations made with 4 g L^{-1} of initial acetate agree with Holt et al. (1984) findings, as butanol final concentration after 300 h reduces as much as 33.2 %, while acetone concentration increases 30 %. Product formation rates show how butanol productivity not only drastically decreases, but its production starts later during fermentation and stops as much as 30 h earlier than in cultures without acetate. This agrees with findings indicating that butyrate re-assimilation is catalysed by the reversible reaction and acetate re-assimilation is catalysed by the CoA-transferase pathway.

11.2 Continuous Stirred Tank Reactor (CSTR)

It is clear that the addition of butyrate to batch fermentation causes a substantial increase in butanol titters. It is, however, also apparent that a batch culture is not the optimal strategy in industrial fermentation because time taken between the end of one culture and the beginning of a new one (sterilization and cleaning) would decrease productivity.

Therefore, butanol production in a CSTR is being taken as the optimal strategy for ABE fermentation, because it can keep production for longer periods, reducing time spent on up and downstream processes (Li et al. 2011a, b).

Further description of continuous culture will be given later in the chapter, while in this section the first working regime studied was using inlet feeding at a dilution rate of 0.04 h^{-1} containing 100 g L^{-1} of glucose and 4 g L^{-1} of butyrate. As previous results indicate that the addition of acetate diminishes butanol formation, this regime was not further studied. Simulations results show that butanol productivity increases 8 % with a final concentration of 3.01 g L^{-1} in the effluent. In terms of the production strategy, the observed increase justifies the addition of butyrate, however further economic studies are need to check if indeed the expense made on butyrate is feasible, because butyrate can be considered an expensive supply.

Simulations were based on a CSTR intended to study the dynamics of the fermentation over wide working regimes, more specifically from dilution rates from 0 to 0.1 h^{-1} and glucose concentrations in the feed from 0 to 200 g L^{-1}. The results show that solvents maximum production rate was between 0.02 to 0.08 h^{-1}, with a maximum butanol production in 0.05 h^{-1}, irrespective of the glucose concentration in the feed, while acid maximum production rates were found into dilution rates higher than 0.05 h^{-1}. This phenomena is typical for ABE fermentation, given that acidogenic and solventogenic phases are related to the growth phase of the culture. It is most likely that in CSTR operated with higher dilution rates, cells were not allowed to remain in the reactor for sufficient time to enter solventogenesis, while the opposite is observed at lower dilution rates (Li et al. 2011a, b). To shorten the

favourable working regions, the cell's catalytic activity was evaluated also, showing that at dilution rates between 0.04 and 0.07 h^{-1} and glucose concentrations between 80 and 120 g L^{-1}, maximum butanol production per unit mass of cells in the reactor was achieved.

11.2.1 CSTR with Two and Three Stages

To further study the strategies under development with CSTRs, further analyses were made considering both two- and three-staged processes. One of the propositions made regarding a two-staged process indicates that high dilution rates in the first reactor are needed to get high acids concentrations which then, taking advantage of *Clostridium's* biphasic metabolism, would be converted to solvents at a posterior stage with low dilution rates (Bahl et al. 1982; Lai and Traxler 1994; Mutschlechner et al. 2000). The results indicate the absence of this phenomenon, as when the conditions set on the first reactor favour acids or solvents production, the second reactor displays an identical internal dynamic, increasing the concentrations of the components produced in the first stage. This behaviour is consistent with experimental evidence, indicating that indeed the conditions set on the first stage become the controlling conditions of the whole process, independently of pH and dilution rate of the posterior stages (Godin and Engasser 1990; Setlhaku et al. 2012).

Propositions made by De Gooijer et al. (1996) indicate that staged processes become favourable when product inhibition is strong, moreover three-staged CSTRs can achieve the closest performance to a CSTR followed by a plug flow reactor. Simulations on staged CSTRs were run in two different conditions, one in which the dilution rates increase in every stage and one with the opposite conditions, being the dilution rates 0.04, 0.06 and 0.08 h^{-1} for the former and 0.04, 0.03 and 0.015 h^{-1} for the latter, both using 100 g L^{-1} of glucose in the feeding of the first stage. Butanol concentration increased in both conditions, as for the system with increasing dilution rates butanol increased 80.56 % from the first stage to the second and from the second to the third 29.78 %, being the final butanol concentrations 2.9, 5.2 and 6.7 g L^{-1} in each stage respectively. This results indicates that there is little improvement with the addition of the third stage, indeed the overall productivity up to the third stage remains the same as if the process only had two stages, being 0.124 g L^{-1} h^{-1} in both scenarios. The system with decreasing dilution rates showed a greater improvement in butanol concentration with an increase of 120.7 % from the first to the second stage and 42.2 % from the second to the third stage, being the final butanol concentrations 6.4 and 9.1 g L^{-1} in the second and third stage respectively. Although the increase in butanol concentrations might be impressive, there is a downside in decreasing the dilution rates because Hydraulic Retention Times (HRTs) of the whole process increase, which result in decreased productivity; for the conditions tested the overall productivity up to the second and third stage were 0.11 and 0.073 g L^{-1} h^{-1}, respectively.

Further simulations show that the productivity when a third stage is added only improves if the total HRT of the whole process is lower than 56 h, nonetheless the

conversion yield from glucose to butanol of the processes with a third stage improves by as much as 60.8 % in systems with HRT's lower than 80 h. Further research is needed to identify which of the conditions has an economic advantage, given that, for example, the conditions with low productivity but higher butanol concentrations could simplify the posterior purification processes up to the point where the production cost is lower than that of a system with higher productivity.

11.3 Fed-Batch Fermentation

Fed-Batch fermentation is a technique used to reduce substrate inhibition, a batch fermentation is run until either a desired substrate concentration or biomass concentration is achieved, then a solution with concentrated substrate is fed at a constant, linear, exponential or intermittent rate. In ABE fermentation, fed batch culture is recommended only with in-line product recovery (Qureshi et al. 1992; Ezeji et al. 2004), due to the strong inhibition effects of butanol, a simple fed-batch would not achieve sufficient productivity to offer any advantages over CSTR operation.

In order to study the behaviour of a fed-batch culture, a theoretical scenario was set up with a 30,000 L bioreactor and constant flow regimes between 34 and 800 L h^{-1} with a glucose feed concentration of 150 g L^{-1}. Maximum productivity was achieved between 200 and 400 L h^{-1}, but it is clear that productivity values reachable through simple fed batch operation are lower, 33 % less, than those of a CSTR. There is, however, an advantage in dealing with a single culture. Fed batch can reach butanol concentrations of 8 g L^{-1} in a single stage, 63 % more than that of a single stage CSTR.

For further analysis, a novel bioreactor configuration, in which the effluent of a CSTR was used as the feed to a fed batch reactor was tested, and the results of both this stage and the one discussed prior to are presented in Figs. 3 and 4. To the

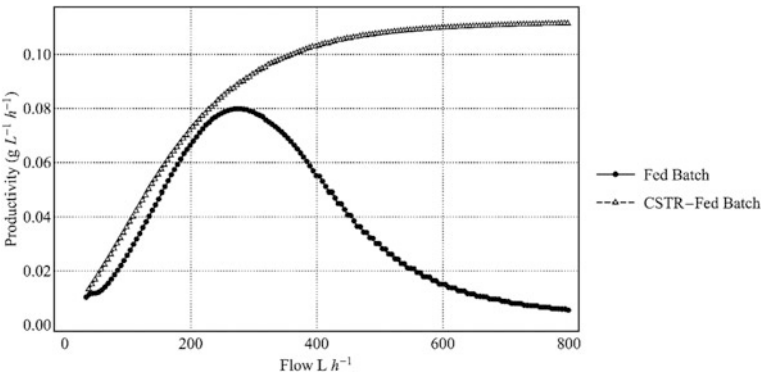

Fig. 3 Theoretical butanol productivity versus feeding flow rate of both a single fed batch reactor and a hybrid system composed of a CSTR and a fed batch fermenters obtained by simulation using the author's kinetic model

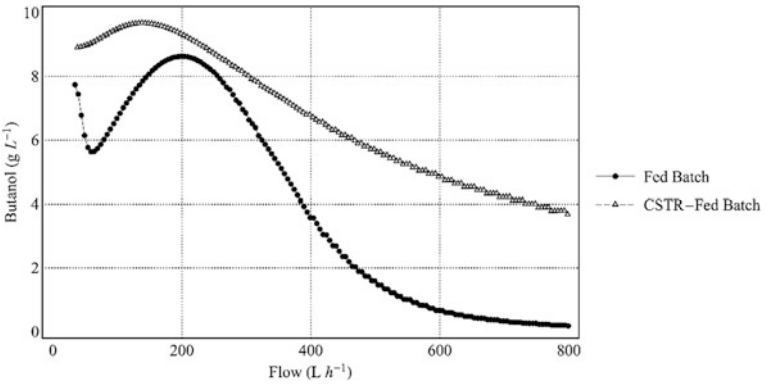

Fig. 4 Final theoretical butanol titter comparison between single fed batch and an hybrid arrange composed of a single stage CSTR and a fed batch reactor obtained via simulations made using the author's kinetic model

authors' knowledge, the work performed by Setlhaku et al. (2012) is the only report of this kind of configuration for ABE fermentation. The study indicates that this system is capable of reaching higher solvent volumetric titters and specific productivity, as well as higher butanol concentration before any product removal technique is applied. The simulations made using the enhanced model proposed here indicate that even though the productivity surpasses that of a single fed batch, the same does not apply for the CSTR. Productivity in this system increases asymptotically with flow, reaching more than 0.12 g L^{-1} h^{-1} in the CSTR at flows higher than 400 L h^{-1}. This sets up two problems, first the difficulty of finding a working region that offers an advantage of some kind, and second, the problems arising from feeding such high fluxes, as it depends on the capacity of the first stage to grow sufficiently before feeding the fed batch, which can lead to scale up troubles. This bioreactor configuration is advantageous in other ways, for instance it reaches 9.6 g L^{-1} of butanol at 0.055 g L^{-1} h^{-1}, which is close to 30 % slower than a three-stage CSTR, moreover the conversion yield of glucose to butanol is 30 % higher (0.24 g But gS^{-1}).

12 Concluding Remarks

As evidenced, a wide arrange of reported methodologies to improve the ABE fermentation economic and environmental feasibility exist, but the current state-of-the-art is still far from providing adequate responses to overcome challenges, especially regarding integration of the technologies with agricultural waste management and treatment. Nonetheless, Clostridial species are solid candidates for consideration as versatile biological systems for transformation of organic residues

into energy compounds and also into commodity chemicals such as CO_2, solvents or even hydrogen. Further study in this area is encouraged and justified.

Further efforts made into the study of the ABE producing processes should incorporate engineering tools like design, optimization and control of bio-systems that could lead to the scale-up of this technology beyond semi-pilot stage. Factors to be considered are: analysis of feedstock availability and costs and the current efforts made into pre-treatment and co-culture techniques to re-evaluate their economic and technical feasibility at large-scale.

References

Alsaker KV, Spitzer TR, Papoutsakis ET (2004) Transcriptional analysis of spo0A overexpression in *Clostridium acetobutylicum* and its effect on the cell's response to butanol stress. J. Bacteriol 186:1959–1971

Antoni D, Zverlov V (2007) Biofuels from microbes. Appl Microbiol Biotechnol 77:23–35

Aro N, Pakula T, Penttila M (2005) Transcriptional regulation of plant cell wall degradation by filamentous fungi. FEMS Microbiol Rev 29(4):719–739. ISSN:0168-6445

Aspinall GO (1959) Structural chemistry of the hemicelluloses. Adv Carbohydr Chem 14:429–468

Atsumi S, Cann AF, Connor MR (2008) Metabolic engineering of Escherichia coli for 1-butanol production. Metab Eng 10:305–311

Badr HR, Toledo R, Hamdy MK (2001) Continuous acetone ethanol butanol fermentation by immobilized cells of *Clostridium acetobutylicum*. Biomass Bioenergy 20:119–132

Bahl H, Andersch W. Gottschalk G (1982) Continuous production of acetone and butanol by *Clostridium acetobutylicum* in a two-stage phosphate limited chemostat. Appl Microbiol Biotechnol 201–205

Bayer EA, Kenig R, Lamed R (1983) Adherence of *Clostridium thermocellum* to cellulose. J Bacteriol 2:818–827

Brown R (2003) Biorenewable resources. Iowa State Press, Blackwell Publishing Co., Iowa

Chang W (2010) Acetone-butanol-ethanol fermentation by engineered *Clostridium beijerinckii* and *Clostridium tyrobutyricum*. Food science and technology PhD Thesis, Ohio State University, USA

Chen C, Blaschek H (1999) Effect of acetate on molecular and physiological aspects of *Clostridium beijerinckii* NCIMB 8052 solvent production and strain degeneration. Appl Environ Microbiol 65(2):499–505

Chen Y, Stevens MA, Zhu Y, Holmes J, Xu H (2013a) Understanding of alkaline pretreatment parameters for corn stover enzymatic saccharification. Biotechnol Biofuels. 6(8)

Chen Y, Zhou T, Liu D, Li An, Xu S, Liu Q, Li B, Ying H (2013b) Production of butanol from glucose and xylose with immobilized cells of *Clostridium acetobutylicum*. Biotechnol Bioprocess Eng 18:234–241

Claassen PAM, Budde MAW, López-Contreras AM (2000) Acetone, butanol and ethanol production from domestic organic waste by solventogenic clostridia. J Mol Microb Biotech 2 (1):39–44

Demirbas A (2009) Progress and recent trends in biodiesel fuels. Energ Convers Manag 50:14–34

Desai RP, Harris LM, Welker NE, Papoutsakis ET (1999) Metabolic flux analysis elucidates the importance of the acid-formation pathways in regulating solvent production by *Clostridium acetobutylicum*. Metab Eng 1(3):206–213

De Gooijer CD, Bakker WAM, Beeftink HH, Tramper J (1996) Bioreactors in series: an overview of design procedures and practical applications. Enzym and Microb Technol 18(3):202–219

Dufey A (2006) Biofuels production, trade and sustainable development: emerging issues. Environmental economics programme/Sustainable markets group, Paper No 2, International Institute for Environment and Development (IIED)

Durán-Padilla VR, Dávila-Vazquez G, Chávez-Vela NA, Tinoco-Valencia JR, Jáuregui-Rincón J (2014) Iron effect on the fermentative metabolism of *Clostridium acetobutylicum* ATCC 824 using cheese whey as substrate. Biofuel Res J 4:129–133

Dürre P (2007) Biobutanol: an attractive biofuel. Biotechnol J 2:1525–1534

Escobar J, Lora S, Venturini O, Yanez E, Castillo E, Almazan O (2008) Biofuels: environment, technology and food security. Renew Sustain Energ Rev 13:1275–1287

Ezeji TC, Qureshi N, Blanschek HP (2004) Acetone butanol ethanol (ABE) production from concentrated substrate: reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping. Appl Microbiol Biotechnol 63:653–658

Gheshlaghi R, Scharer JM, Moo-Young M, Chou CP (2009) Metabolic pathways of clostridia for producing butanol. Biotechnol Adv 27(6):764–781

Gírio FM, Fonseca C, Carvalheiro F, Duarte LC, Marques S, Bogel-Łukasik R (2010) Hemicelluloses for fuel ethanol: a review. Bioresour Technol 101(13):4775–4800. ISSN:09608524

Godin G, Engasser M (1990) Two-stage continuous fermentation of *Clostridium acetobutylicum* : effects of pH and dilutio rate. Appl Microbiol Biotechnol 269–273

Gomez LD, Clare GS, McQueen-Mason J (2008) Sustainable liquid biofuels from biomass: the writing's on the walls. New Phytol 178:473–485

Harris EE (1949) Wood saccharification, advances in carbohydrate chemistry, vol IV. Academic Press Inc, New York

Haus S, Jabbari S, Millat T, Janssen H, Fischer R-J, Bahl H, King JR, Wolkenhauer O (2011) A systems biology approach to investigate the effect of pH-induced gene regulation on solvent production by *Clostridium acetobutylicum* in continuous culture. BMC Syst Biol 5:5–10

Holt RA, Stephens GM, Morris JG (1984) Production of solvents by *Clostridium acetobutylicum* cultures maintained at neutral pH. Appl Environ Microbiol 48(6):1166–1170

Hüsemann MH, Papoutsakis ET (1988) Solventogenesis in *Clostridium acetobutylicum* fermentations related to carboxylic acid and proton concentrations. Biotechnol Bioeng 32(7):843–852

Keis S, Shaheen R, Jones D (2001) Emended descriptions of *Clostridium acetobutylicum* and *Clostridium beijerinckii*, and descriptions of *Clostridium saccharoperbutylacetonicum* sp. nov. and *Clostridium saccharobutylicum* sp. nov. Int J Syst Evol Microbiol 51:2095–2103

Klemn D, Heublein B, Fink H-P, Bohn A (2005) Cellulose: fascinating biopolymer and sustainable raw material. Angew Chem Int Ed 44:3358–3393

Kobayashi T, Mitachi K, Kikuchi H (1962) Main-hydrolysis of White Birch Chips Wetted with Sulfuric Acid Solution of Medium Strength Using a Through Drying Operation. Agr Biol Chem 26(6):371–377

Lai M-C, Traxler RW (1994) A coupled two-stage continuous fermentation for solvent production by *Clostridium acetobutylicum*. Enzym Microbial Technology 16(12):1021–1025

Lamed R, Setter E, Kenig R, Bayer EA (1983a) The cellulosome: a discrete cell surface organelle of *Clostridium thermocellum* which exhibits separate antigenic, cellulose-binding and various cellulolytic activities. Biotechnol Bioeng 13:63–181. ISSN:0006-3592

Lamed R, Setter E, Bayer EA (1983b) Characterization of a cellulose-binding, cellulase-containing complex in *Clostridium thermocellum*. J Bacteriol 2:828–836

Lee SY, Park JH, Jang SH, Nielsen LK, Kim J, Jung KS (2008a) Fermentative butanol production by clostridia. Biotechnol Bioeng 101–2:209–228

Lee S, Cho MO, Park CH, Chung Y, Kim JH (2008b) Continuous Butanol production using suspended and immobilized *Clostridium beijerinckii* NCIMB 8052 with supplementary butyrate. Energy Fuels 13:3459–3464

Lehmann D, Hönicke D, Ehrenreich A, Schmidt M, Weuster-Botz D, Bahl H, Lütke-Eversloh T (2012) Modifying the product pattern of *Clostridium acetobutylicum*: physiological effects of disrupting the acetate and acetone formation pathways. Appl Microbiol Biotechnol 94(3): 743–754

Li R-D, Li Y-Y, Lu L-Y, Ren C, Li Y-X, Liu L (2011a) An improved kinetic model for the acetone-butanol-ethanol pathway of *Clostridium acetobutylicum* and model-based perturbation analysis. BMC Syst Biol 5(Suppl 1):S12

Li S-Y, Srivastava R, Suib SL, Li Y, Parnas RS (2011b) Performance of batch, fed-batch, and continuous A-B-E fermentation with pH-control. Bioresour Technol 102(5):4241–4250

Liew ST, Arbakariya A, Rosfarizan M, Raha AR (2005) Production of solvent (acetone-butanol-ethanol) in continuous fermentation by *Clostridium saccharobutylicum* DSM13864 using gelatinised sago starch as a carbon source. Malays J Microbiol 2(2):42–45

Liu H, Wang G, Zhang J (2013) In: Fang Z (ed), The promising fuel-biobutanol, liquid, gaseous and solid biofuels—conversion techniques, ISBN:978-953-51-1050-7, InTech, doi:10.5772/52535. Available from: http://www.intechopen.com/books/liquid-gaseous-and-solid-biofuels-conversion-techniques/the-promising-fuel-biobutanol

Lo YC, Bai MD, Chen WM, Chang JS (2008) Cellulosic hydrogen production with a sequencing bacterial hydrolysis and dark fermentation strategy. Bioresour Technol 99(17):8299–8303

López-Contreras AM (2001) *Clostridium beijerinckii* cells expressing Neocallimastix patriciarum glycoside hydrolases show enhanced lichenan utilization and solvent production. Appl Environ Microbiol 67:5127–5133

Maddipati P, Atiyeh HK, Bellmer DD, Huhnke RL (2011) Ethanol production from syngas by *Clostridium* strain P11 using corn steep liquor as a nutrient replacement to yeast extract. Bioresour Technol 102(11):6494–6501

Martín C, Marcet M, Almazán O, Jönsson LJ (2007) Adaptation of a recombinant xylose-utilizing saccharomyces cerevisiae strain to a sugarcane bagasse hydrolysate with high content of fermentation inhibitors. Bioresour Technol 98(9):1767–1773. ISSN:0960-8524

Martınez AT, Ruiz-Dueñas FJ, Martínez MJ, Del Río JC, Gutiérrez A (2009) Enzymatic delignification of plant cell wall: from nature to mill. Curr Opin Biotechnol 20(3):348–357. ISSN:0958-1669

Mayank R, Ranjan A, Moholkar VS (2012) Mathematical models of ABE fermentation: review and analysis. Crit Rev Biotechnol 1–29

McNew K, Griffith D (2005) Measuring the impact of ethanol plants on localgrain prices. Rev Agric Econ 27:164–180

Melaina MW, Heath G, Sandor D, Steward D, Vimmerstedt L, Warner E, Webster KW (2013) Alternative fuel infrastructure expansion: costs, resources, production capacity, and retail availability for low-carbon scenarios. Transportation energy futures series. 101 Prepared for the U.S. Department of Energy by National Renewable Energy Laboratory, Golden, CO. DOE/GO-102013-3710

Millat T, Voigt C, Janssen H, Cooksley CM, Winzer K, Minton NP, Wolkenhauer O (2014) Coenzyme A-transferase-independent butyrate re-assimilation in *Clostridium acetobutylicum*-evidence from a mathematical model. Appl Microbiol Biotechnol 98:9059–9072

Mutschlechner O, Swoboda H, Gapes JR (2000) Continuous two-stage ABE-fermentation using *Clostridium beijerinckii* NRRL B592 operating with a growth rate in the first stage vessel close to its maximal value. J Mol Microbiol Biotechnol 2:101–105

Mužíková Z, Šimáček P, Pospíšil M, Šebor G (2014) Density, viscosity and water phase stability of 1-butanol and gasoline blends. J Fuels 2014, ID 459287

Naik SN, Goud VV, Rout PK, Dalai AK (2010) Production of first and second generation biofuels: a comprehensive review. Renew Sustain Energy Rev 14:578–597

Nakayama S, Kiyoshi K, Kadokura T, Nakazato A (2011) Butanol production from crystalline cellulose by cocultured *Clostridium thermocellum* and *Clostridium saccharoperbutylacetonicum* N1-4. Appl Env Microbiol 77(18):6470–6475

Napoli F, Olivieri G, Russo M, Marzocchella A, Salatino P (2011) Continuous lactose fermentation by *Clostridium acetobutylicum*—assessment of acidogenesis kinetics. Bioresour Technol 102–2:1608–1614

Nordon RE, Craig SJ, Foong FC (2009) Molecular engineering of the cellulosome complex for affinity and bioenergy applications. Biotechnol Lett 31(4):465–476. ISSN:1573-6776

Ogden J, Williams RH, Larson ED (2004) Societal lifecycle costs of cars with alternative fuels/engines. Energy Policy 32(1):7–27
Pandey A (2003) Solid-state fermentation. Biochem Eng J 13(2–3):81–84. ISSN:1369-703X
Papoutsakis ET, Rice U (1984) Equations and calculations for fermentations of butyric acid bacteria. Biotechnol Bioeng 26(2):174–187
Peralta-Yahya PP, Zhang F, del Cardayre SB, Keasling JD (2012) Microbial engineering for the production of advanced biofuels. Nature 488:320–328
Pfromm PH, Amanor-Boadu V, Nelson R, Vadlani P, Madl R (2010) Bio-butanol versus bio-ethanol: a technical and economic assessment for corn and switchgrass fermented by yeast or *Clostridium acetobutylicum*. Biomass Bioenergy 34:515–524
Pimentel D, Patzek TW, Gerald C (2007) Ethanol production: energy, economic, and environmental losses. Rev Environ Contam Toxicol 189:25–41
Qureshi N, Maddox IS, Friedl A (1992) Application of continuous substrate feeding to the ABE fermentation: relief of product inhibition using extraction, perstraction, stripping, and pervaporation. Biotechnol Prog 8:382–390
Qureshi N, Saha BCJ, Cotta MA (2007) Butanol production from wheat stray hydrolysate using *Clostridium beijerinckii*. Bioprocess Biosyst Eng 30:419–427
Qureshi N, Ezeji TC, Ebener J, Dien BS, Cotta MA, Blaschek HP (2008) Butanol production by *Clostridium beijerinckii*. Part I. Use of acid and enzyme hydrolyzed corn fiber. Bioresour Technol 99:5915–5922
Qureshi N, Saha BC, Dien B, Hector RE, Cotta MA (2010) Production of butanol (a biofuel) from agricultural residues: Part I. Use of barley straw hydrolysate. Biomass Bioenergy 34:559–565
Saha BC, Iten LB, Cotta MA, Wu V (2005) Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. Process Biochem 40:3693–3700
Sandgren M, Hiberg JS (2005) Structural and biochemical studies of gh family 12 cellulases: improved thermal stability and ligand complexes. Prog Biophys Mol Biol 89(3):246–291. ISSN:0079-6107
Scheller HV, Ulvskov P (2010) Hemicelluloses. Ann Rev Plant Biol 61:263–89. ISSN:1543-5008
Setlhaku M, Brunberg S, Villa A, Wichmann R (2012) Improvement in the bioreactor specific productivity by coupling continuous reactor with repeated fed-batch reactor for acetone—butanol—ethanol production. J Biotechnol 161(2):147–152. doi:10.1016/j.jbiotec.2012.04.004
Sharma B, Ingalls RG, Jones CL, Khanchi A (2013) Biomass supply chaindesign and analysis: basis, overview, modeling, challenges, and future. Renew Sustain Energy Rev 24:608–627
Shinto H, Tashiro Y, Yamashita N, Kobayashi G, Sekiguchi T, Hanai T, Kuriya Y, Okamoto M, Sonomoto K (2007) Kinetic modeling and sensitivity analysis of acetone-butanol-ethanol production. J Biotechnol 131(1):45–56
Singh R, Kapoor V, Kumar V (2012) Utilization of agro-industrial wastes for the simultaneous production of amylase and xylanase by thermophilic actinomycetes. Braz J Microbiol 1545–1552
Soni BK, Das K, Ghose TK (1982) Bioconversion of agro-wastes into acetone butanol. Biotechnol Lett 4(1):19–22
Sun Y, Cheng JY (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83(1):1–11
Terracciano JS, Kashket ER (1986) Intracellular conditions required for initiation of solvent production by *Clostridium acetobutylicum*. Appl Environ Microbiol 52(1):86–91
Thorn GJ, King JR, Jabbari S (2013) pH-induced gene regulation of solvent production by *Clostridium acetobutylicum* in continuous culture: parameter estimation and sporulation modelling. Math Biosci 241(2):149–166
Tran HTM, Cheirsilp B, Hodgson B, Umsakul K (2010) Potential use of *Bacillus subtilis* in a co-culture with *Clostridium butylicum* for acetone-butanol-ethanol production from cassava starch. Biochem Eng 48:260–267
Tummala SB, Welker NE, Papoutsaks ET (2003) Design of antisense RNA constructs for downregulation of the acetone formation pathway of *Clostridium acetobutylicum*. J Bacteriol 185:1923–1934

Uyeda K, Rabinowitz JC (1971) Pyruvate-ferredoxin oxidoreductase. IV. Studies on the reaction mechanism. J Bacteriol Chem 246:3120–3125

Vamvuka D, Kastanaki E, Grammelis P, Kakaras E (2003) Pyrolysis characteristics and kinetics of biomass residuals mixtures with poor coal. Fuel 82:1949

Velázquez-Sánchez HI, Montes-Horcasitas MC, Aguilar-López R (2014) Development of a phenomenological kinetic model for butanol productiong using *Clostridium Beijeinckii*. Revista Mexicana de Ingeniería Química 13(1):103–112

Wang Y, Li X, Blaschek HP (2013) Effects of supplementary butyrate on butanol production and the metabolic switch in *Clostridium beijerinckii* NCIMB 8052: genome-wide transcriptional analysis with RNA-Seq. Biotechnol Biofuels 6(1):138

Wen Z, Wu M, Lin Y, Yang L, Lin J, Cen P (2014) Artifficial symbiosis for acetone-butanol-ethanol (ABE) fermentation from alkali extracted deshelled corn cobs by co-culture of Clostridium beijerinckii and Clostridium cellulovorans. Microb Cell Fact 13: 92–103

Wietzke M, Bahl H (2012) The redox-sensing protein Rex, a transcriptional regulator of solventogenesis in *Clostridium acetobutylicum*. Appl Microbiol Biotechnol 96(3):749–761

Yuea D, Youa F, Snyder SW (2014) Biomass-to-bioenergy and biofuel supply chain optimization: overview, key issues and challenges. Comput Chem Eng 66:36–56

Zhao Y, Tomas C (2005) Intracellular butyryl phosphate and acetyl phosphate concentrations in *Clostridium acetobutylicum* and their implications for solvent formation. Appl Environ Microbiol 71(1):530–537

Zheng Y, Li L, Xian M, Ma Y, Yang J, Xu X, He D (2009) Problems with the microbial production of butanol. J Ind Microbiol Biotechnol 36:1127–1138

Zverlov V, Berezina O, Velikodvorskaya GA, Schwarz WH (2006) Bacterial acetone and butanol production by industrial fermentation in the Soviet Union: use of hydrolyzed agricultural waste for biorefinery. Appl Microbiol Biotechnol 71:587–597