



Optimum utilization of *Clostridia* species towards biofuel production

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Abstract

Global increasing stipulates for the production of renewable fuels due to massive utilization of readily available fossil fuel, more interests in microbial production of biofuels are generated. This opened great opportunities to the biologists, because anaerobic bacteria particularly *Clostridium* species are capable of converting carbohydrates into a variety of solvents such as acetone, butanol, ethanol and more the like. The review provided ample sources of information with regards to the potentialities of *Clostridium* species towards production of biofuels. The classification of *Clostridium* species into pathogenic and non-pathogenic, and those capable of biofuel production has been summarized. Typical metabolic processes responsible for transforming biomass into various biofuels have been highlighted. Utilization of agricultural wastes as substrates towards biofuel production was equally highlighted. Various carbon sources and some *Clostridium* species exploited for biofuel production were summarized. The review also provided some of the factors that influenced the biofuel production.

Keywords: optimum, clostridium, biofuel

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Introduction

Louis Pasteur was the first to discover microorganisms in 1861. Nevertheless, microorganisms producing biofuels were first discovered in the early of 1920s by Chaim Weizmann. He discovered the *Clostridium acetobutylicum* (a spore forming, rod shaped, obligate anaerobe and gram-positive bacteria) and was reported to be employed in large scale fermentation by using sugar and starchy grains for production of acetone and butanol (Johnson et al., 1997). However, the *Clostridium acetobutylicum*, in nature, produces Acetone, Butanol, and Ethanol (ABE) in a ratio of 3:6:1. The initial

production plants for the production of ABE fermentation were developed due to the First World War dependent demand of acetone for a cordite manufacture, during which biofuels were only an unwanted byproduct (Tina and Hubert, 2011) and later on become a vital product as a result of high reliance on fossil fuel.

It is also important to note here that *Clostridium* species are classified into pathogenic and non-pathogenic. The non-pathogenic are those capable of producing biofuels and are also of two types. The acetic acids, butyric acid, and gasses (H₂ and CO₂) producing group like

C. butyricum, and those that produce acids (*C. acetilybutylicum*) and are capable of converting them to solvents, acetone, butanol and ethanol (Morris, 1994). Added to that, many more species of *Clostridia* have been used purposely to produce organic acids and solvents by acting up on long chain sugars into simple and consumable sugars (monosaccharide). The broad classification of the *Clostridium* species based on harmful and beneficial and their end products is presented in table 1.

Similarly, the genus *Clostridia* holds an enormous variety of bacteria capable of producing biofuels like *C. acetobutylicum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum*, *C. saccharoacetobutylicum*, *C. aurantibutyricum*, *C. pasteurianum*, *C. sporogenes*, *C. cadaveris*, and *C. tetanomorphum*). A research conducted by Huang (2010) showed that *C. acetobutylicum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum*, and *C. saccharoacetobutylicum* demonstrated a significant activity of forming butanol with higher yields. Previously, it was believed that *C. acetobutylicum* was the only type that proposed to be used for ABE fermentation. Nevertheless, all through 1990's some other species were recognized namely *C. beijerinckii*, *C. saccharoperbutylacetonicum* and *C. saccharobutylicum* along with *C. acetobutylicum*. Afterward, different strains of the above species have been evaluated and reclassified through the use of a gene sequencing technique named 16S rRNA (Johnson et al., 1997; Winzer et al., 2000). Nevertheless, not only *Clostridium* species are involved in biofuel production. Well-known and studied microorganisms such as *E. coli* provide an excellent scientific stand for biofuel production (Tina and Hubert, 2011).

Metabolism processes

Lignocellulosic materials and other feedstock can be transformed into solvents through hydrolysis and succeeding processes of fermentation. This transformation produces some varieties of simple sugar like, glucose, xylose and arabinose which then go through a glycolysis and fermentation process to obtain the solvents (Thaddeus et al., 2007a), particularly biobutanol and acetone, as shown (Fig. 1). From the figure 1 biomass metabolism by *Clostridium* specie commences through a biomass and lignocelluloses pretreatment; then move to starch hydrolysis by enzyme activities, some of the enzymes involved include α -amylase, β -amylase, pullulanase, and glucoamylase; it then followed a cellulose hydrolysis by some enzymes called cellulases B-glucosidase; eventually the process concluded the extracellular part through a hemicellulose hydrolysis (Thaddeus et al., 2007b). Next to this is the intracellular part of the process which is referred to as solventogenetic and acidogenetic phases. These phases are responsible for the production of biobutanol, acetone, ethanol (Solventogenesis), acetic acid, and butyric acid (Acidogenesis).

The enzymes involved in the process are designated by numbers as shown accordingly: Enzymes responsible for glycolysis like hexokinase, glucose phosphate isomerase, phosphofructokinase, and fructose diphosphate aldolase 1; Pyruvate ferredoxinoxidoreductase 2; acetaldehyde dehydrogenase 3; ethanol dehydrogenase 4; phosphate acetyltransferase 5; acetate kinase 6; acetyl-CoA acetyltransferase 7; 3-hydroxybutyryl-CoA dehydrogenase 8; acetate/butyrate, acetoacetyl-CoA, CoA-transferase 9; acetoacetate decarboxylase 10; crotonase 11; butyryl-CoA dehydrogenase 12, phosphate butyltransferase 13; butyrate

kinase 14; butyraldehyde dehydrogenase 15; butanol dehydrogenase 16; and lastly hydrogenase 17 (Ezeji et al., 2007; Huang et al., 2010).

Anaerobic digestion processes

As the hydrolysis of the biomass is accomplished,

hexose sugars are metabolized by means of

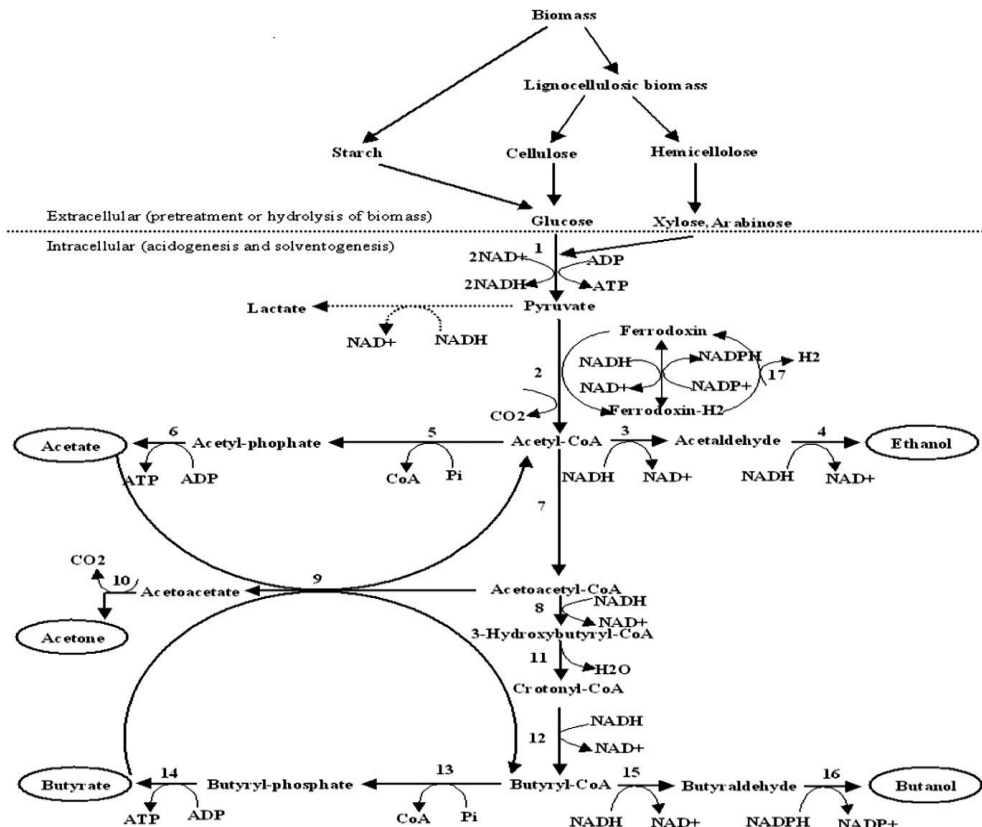
Table 1. Beneficial and harmful *Clostridium* species and their end products

Species categories and their end products					
A			B		
Toxic	Products	References	Non-toxic	Products	References
<i>C. botulinum</i>	Toxins	Andreesen et al. (1989)	<i>C. butyricum</i>	Acetic acid, Butyric acid and gases (H ₂ and CO ₂)	Andel et al. (1985)
<i>C. perferingens</i>			<i>C. beijerinckii</i>	Acetic and butyric acids, H ₂ and CO ₂ , butanol, ethanol, and isopropanol	Green and Stephens (1996)
<i>C. histolyticum</i>			<i>C. acetobutylicum</i>	Acetic and butyric acids, H ₂ and CO ₂ , Acetone, Butanol, and Ethanol	Qureshi et al., (1992), Girbal and Soucaille (1998)
<i>C. tetani</i>			<i>C. aurantibutyricum</i>	Acetic and butyric acids H ₂ and CO ₂ gases, Butanol and Ethanol	Somrutai et al. (1996)
			<i>C. tetenomorphum</i>	Acetic and butyric acids H ₂ and CO ₂ gases, Butanol and Ethanol	Gottwald et al. (1984)
			<i>C. tyrobutyricum</i>		

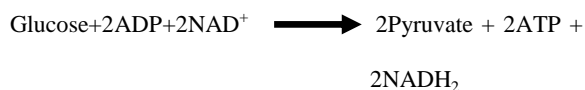
Table 2. List of different carbon sources

Carbon sources	Microorganism	References
Sago Starch	<i>Clostridium saccharobutylicum</i> P262	Madihah et.al., 2008
Corn	<i>Clostridium beijerinckii</i> BA101	Qureshi and Blaschek, 2001
Potato	<i>Clostridium acetobutylicum</i> DSM 1731	Gutierrez et al., 1997
Maize stalk juice	<i>Clostridium beijerinckii</i> NCMB 8052	Yi and Blaschek, 2011

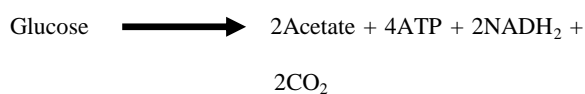
Fig. 1. Metabolism of Biomass by Solventogenic *Clostridium* (Gheshlaghi et al., 2009)



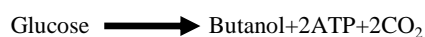
glycolysis to form a mole of hexose sugars which are further transformed into two moles of pyruvate, with the total production of two moles of adenosine triphosphate (ATP) and two moles of Nicotinamide Adenine Dinucleotide (NADH). Moreover, pentose sugars became transformed into glucose through pentose phosphate pathway resulting in the production of fructose-6-phosphate and glyceraldehyde-3-phosphate. All through during acidogenesis, the bacteria grow exponentially where acetic acid and butyric acid are produced together with formation of ATP. These two acids are formed from the acetyl-CoA and butyryl CoA synthesized by the enzyme phosphate acetyltransferase and acetate kinase, so also phosphate butyryltransferase and butyrate kinase. However, in solventogenesis phase, the cell grows at stationary phase, in which the organic acids are then synthesized again for acetone, butanol and ethanol production. This pathway is responsible for the production of acetylaldehyde, acetoacetate, and butyraldehyde. However, the reduction of butyryl-CoA to butanol is performed by enzymes called butyraldehyde dehydrogenase and butanol dehydrogenase (Gheshlaghi et al., 2009). The processes involved starting from glycolysis until solvents production is summarized below,



Acidogenesis



Solventogenesis



Biomass as substrate for biofuel production

Biomass for fermentation process is most responsible part on the basis of its feasibility in an economic wise (Qureshi and Blaschek, 2000). On the basis of utilization of substrate, biofuels were classified into first and second generations. In the first generation, raw materials used were sugarcane and cereal grains while in the second generation, lignocellulosic materials (Agriculture and forest wastes) were used as substrates. It should be noted that the raw materials used for the first generation were food competitive, while the second generation were non-edible materials (Naik et al., 2010) and therefore are non-food competitive. Currently, there is an increased focus on second generation biofuel due to the availability of cheaper raw materials (Hoekman, 2009) and are not foods competitive. Durre (1998) pointed out that the traditional attempts in utilizing cereal grains and sugar as substrate in the ABE fermentation process for large scale production were promoted by the availability of the raw materials and the massive necessity of fermentation products. However, these substrates utilization was covered due to price hikers, so also contributed to food shortages (Pfromm et al., 2010). This hesitation imposed to explore inexpensive and non-food competitive raw materials for ABE fermentation. Fortunately, it is found that various carbohydrates such as glucose, fructose, mannose, sucrose, lactose, starch, and dextrin were consumed totally by *Clostridium* species, while galactose, xylose, arabinose, raffinose, melezitose, inulin and minnitol were partially utilized. Studies have revealed that

xylose and arabinose could also be completely fermented by most *Clostridium* species. However, these organisms were unable to consume trehalose, rhamnose, melibiose, and glycerol (Jones and Woods, 1986). It is well understood that raw materials containing above mentioned simple sugars, because they are less expensive and relatively abundant, can maintain or improve the economics of ABE fermentation (Zhang et al., 2010). Moreover, nowadays, it is known that *Clostridium* species can consume other cheaper alternative substrates such as, lignocellulosic materials due to their saccharolytic capability (Ezeji et al., 2007). However, acidic or enzymatic hydrolysis of lignocellulosic materials has been crucial to convert into simple sugars before being used as substrates in ABE fermentation. The lignocellulosic biomass is among the most abundant renewable resource globally for biofuel production (Antoni et al., 2007). For instance, developing countries like India generates over 370 million tonnes of industrial raw materials per annum from plants, rice husk from rice mills, sawdust from saw mills, bagasse from sugar mills etc. (Chauhan, 2010). To mention a few more, as a result of more insights on the significance of biobutanol production, a lot of researches were undertaken on the fermentation processes to produce biofuel using different types of biomass as shown in Table 2.

Factors influencing biofuel production

Acetone-Butanol-Ethanol (ABE) production through fermentation is now attracting people minds, however the processes involved are highly complex, because it is greatly influenced by many different factors at all angles. For example, proper pH control is crucial for the process to shift to solventogenesis and produce a high butanol yield (Jones and Woods, 1986); while sugar concentration both

low and high in a raw material used as substrates could lead to reduced microbial growth (and unfavorable solvent production), and substrate inhibition (which may inhibit cell growth and cause failure of fermentation); suitable agitation rate can facilitate mixing of the substrates and products, enhancing substrate accessibility and products distribution, but high agitation may adversely impact the fermentation, and lead to unnecessary waste of energy coupled with poor industrial economics (Yi et al., 2011). Temperature is equally among the factors that tremendously influence biobutanol production. It was clearly stated by Madi et al. (1987) that temperature of a medium during fermentation exercise influenced the total yield, the ratios of solvents, and the rate at which solvents are produced. They also pointed out that optimum temperature responsible for solventogenesis by some of the *Clostridium* species was nearly 30°C. The medium constituents are also among the factors that show a high impact on both solvents and acid production. The microbial cells physiological appearance and biosynthetic mechanism can easily be altered by amendment of the chemical composition of the nutrients found in the medium (Welsh and Veliky, 1984). Therefore, apposite choice of substrates sources both organic and inorganic (like nitrogen, carbon, minerals, vitamins) is highly crucial in optimizing the production of acids and solvents. Organic nitrogen sources constitutes vital compounds like protein, yeast extract, amino acid, and glutamic acid, which are all excellent compounds in facilitating microbial growth when supplemented in a culture medium (Abou-Zied and Yassein, 1976) and this could be due to availability of vitamins and other growth initiators present in the organic nitrogen. However, combining certain organic and inorganic nitrogen sources could facilitate the microbial

rate of growth, utilization of substrates and Solventogenesis (Welsh et al., 1987). Another vital factor that greatly affects acidogenesis, solventogenesis, and microbial cell growth is the level of nitrogen and also the ratios of carbon to nitrogen, for exceptional microbial cell growth favoring Solventogenesis were usually found at a low ratio (less than 0.2) of carbon to nitrogen. However, when the ratio is high (greater than 0.2), nitrogen furnish constraint diminish both substrate consumption and cell growth, thereby altering the shifting of the process to solventogenesis although there is abundance sugar in the culture medium (Lai and Traxler, 1984).

Conclusion

Biofuel now became highly attractive and a best substitute to fossil fuel. The values and applications of biofuels into different aspects of industrial and domestic processes can ever be emphasized due to the availability, cheap, and optimum utilization of waste biomass which make them easier and equally open opportunities for biologist to explore the fermentation processes. Consequently, series of researches have been carried out to raise the level of biofuel production through utilizing different Clostridial strains and substrates (both carbon and nitrogen sources, including organic and in-organic).

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