Review Article

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Acetic acid bacteria: A group of potential microorganisms for conversions of biological wastes

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ABSTRACT

Biological wastes, occurred during food and other industrial processes, have feasibility to be promising substrates for microbial cultivation. The wastes could be value-added by the microbial conversion. Acetic acid bacteria (AAB) are well-known as potential microorganisms for producing high-value products using low-value substrates. Oxidative cultivation processes of AAB enable the use of biological wastes to produce high-value chemicals such as acetic acid, dihydroxyacetone, and bacterial cellulose which could be further used in many applications. *Acetobacter* and *Gluconobacter* are potential candidates for the purposes. This review aims to provide potential species of AAB with capability of using biological wastes as substrate for producing high value products.

Keywords: Acetic acid bacteria; *Acetobacter*; *Gluconobacter*; Biological wastes; Bioconversion

1. INTRODUCTION

Biological by-products or wastes occurred during industrial processes could be potential sources for microbial cultivation. The wastes are still rich in energy source that can be consumed by microorganisms. Many wastes or by-products such as molasses, crude glycerol, and other wastes obtained from food industries were used as substrates for microorganisms in order to produce valuable products or chemicals. Molasses which is a waste from sugar industry contains up to 77% of sugars (Pérez, 1995). Crude glycerol a by-product from biodiesel production process contains 80% of glycerol (da Silva et al., 2009). Almost 100% of sugars and glycerol contained in molasses and crude glycerol, respectively, could be consumed by microorganism. However, trace amount of impurity such as acid and alcohol contained in the substrates could slightly limit the yield of the bioconversion (da Silva et al., 2009; Hashizume et al., 1967). Other biological wastes such as bagasse and fruit peel still contain valuable carbon source which can be used as substrates for microbial cultivation (Cheng et al., 2017; Preethi et al., 2017; Yanti et al., 2017).

Microbial conversion is a promising method for producing useful products. Microbial conversion is considered as a green technology which does not release hazardous wastes into the environment. Microorganisms consume substrates by either oxidative and/or reductive pathways. For example, glycerol could be either oxidatively metabolized into dihydroxyacetone by acetic acid bacteria (AAB) or reductively metabolized into 3-hydroxypropionaldehyde by anaerobic bacteria (da Silva et al., 2009). Many industries have applied the microbial conversion techniques to treat or value

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added biological wastes. Bacteria, yeasts and fungi have been used for consuming biological wastes in many purposes such as waste water treatment (Quan et al., 2005), bio-ethanol production (Park et al., 2010; Ha et al., 2011), and enzymes production (Wang et al., 2008; Dhillon et al., 2011; Santis-Navarro et al., 2011).

AAB is a promising microorganism that could be applied for treating or converting the biological wastes into high-value products. AAB is capable of oxidizing various carbon sources (Mamlouk and Gullo, 2013). The bacteria have been used in many industrial processes such as for the production of vinegar, fermented beverages, cocoa, cellulose, and other bioconversion products (Raspor and Goranovic, 2008). *Acetobacter*, *Gluconobacter*, and *Gluconacetobacter* are the major three genera of AAB that have been used in several industrial processes. *Acetobacter aceti* has been used for the vinegar production (Raspor et al., 2008) and *Gluconobacter oxydans* has been cultivated for dihydroxyacetone production (Zhou et al., 2016). Other species of AAB including *A. xylinum* (Cheng et al., 2017) which was reclassified as *Komagataeibacter xylinus*, *G. frateurii* (Poljungreed and Boonyarattanakalin, 2017), *Gluconacetobacter hansenii* (Costa et al., 2017) and *Gluconacetobacter diazophicus* (Eskin et al., 2014; Costa et al., 2017) were also investigated for their feasibility in industrial uses. *Komagataeibacter xylinus* was cultured using bagasse, molasses, and sago liquid waste as substrates in order to produce bacterial cellulose. *G. frateurii* was used to produce dihydroxyacetone using glycerol as a sole carbon source. *Gluconacetobacter hansenii* was also investigated to produce bacterial cellulose and *Gluconacetobacter diazophicus* was isolated from sugar cane in order to investigate for indoleacetic acid production.

This paper aims to present potential AAB that have capability for using biological wastes including molasses, crude glycerol, and other biological wastes as substrates for high-value product generation. Metabolic pathways of AAB to produce each products are described. The paper discusses in depth on the nutrients contained in the biological wastes that are preferable for the microbial cultivation. High value products or chemicals that can be produced by the microbial cultivations using biological wastes as substrates are also revealed in this paper. This article will benefit as a guidance for treating or using biological wastes or by-products of industrial processes in order to decrease cost of the industrial production processes.

2. FEASIBILITY OF BIOLOGICAL WASTES AS SUBSTRATE FOR MICROBIAL CULTIVATION

As biological wastes occurred during industrial production processes still contain energy source, the wastes could be consumed by microorganisms and converted into value-added products. Molasses, crude glycerol, and other biological wastes which are released from either food or other industries are promising choices for the purpose because the biological wastes contain reasonable amount of energy source for microbial cultivation. Molasses and sugarcane bagasse have been normally employed as animal feed, fuel, paper, and cardboard procurement. However, the by-products could be used as substrate for ethanol, lactic acid, citric acid, and sorbitol productions by bioconversions (Veana et al., 2014). Carbon source is a major energy source remaining in the biological wastes. Since major elements required for the microbial growth or fermentation processes are carbon, oxygen, nitrogen, and hydrogen (Srivastava and Srivastava, 2003), carbon source plays an important role on either growth or other activities of microorganisms. The following information reveals major components of the biological wastes. The information also present pretreating process required for particular substrate that contains complex carbon source which cannot be directly consumed by microorganisms.

Molasses, a by-product from sugar industry, contains up to 48 - 77% of carbohydrate (Hashizume et al., 1967; Heuzé et al., 2015). Sugar is a basic energy

substrate for living organisms. Sugar remaining in molasses is mostly sucrose (Pérez, 1995). Sucrose could be consumed and metabolized through glycolysis pathway in order to produce pyruvate which is the substrate for further metabolism of organism. Throughout the metabolism of a sucrose molecule, 76 molecules of energy for living organism in term of adenosine triphosphate (ATP) could be obtained (Campbell and Reece, 2001).

Beside carbon source, molasses also contains trace amounts of nitrogenous compounds and inorganic constituents including K_2O, CaO, MgO, and SO_3 which could be preferred for microbial cultivation (Hubert, 1963). Hence, molasses is a very practical substrate option for further microbial based production.

Sugarcane bagasse, a fibrous by-product from sugar production, contains mostly carbohydrate fiber including 50% of cellulose, 25% of hemicellulose, and 25% of lignin (Wright et al., 2016). Sugarcane bagasse can be hydrolyzed by either enzymatic activity of microorganism (Garg and Neelakantan, 1982) or acid hydrolysis (Cheng et al., 2017) in order to produce sugars which could be used as substrates for microbial production. Sugarcane bagasse has been investigated as a substrate for microbial productions such as protein from mushrooms (Poonam et al., 1987; El-Sayed et al., 1994), and bioethanol (Hernawan et al., 2017). These evidences suggest that sugarcane bagasse could be a practical substrate for microbial cultivation.

Crude glycerol, a by-product from biodiesel production, is another promising substrate for microbial cultivation aspect. Normally, glycerol is used in many applications such as pharmaceutical, food, cosmetic, and personal care products; however, purified glycerol is required for the applications (da Silva et al., 2009). The crude glycerol contains up to 80% of glycerol which could be consumed by microorganisms through either oxidative or reductive pathways (da Silva et al., 2009). For oxidative pathway, glycerol could be converted into dihydroxyacetone phosphate (DHA-P) and then further metabolized through glycolysis and Krebs cycle (Campbell et al., 2001). For reductive pathway, glycerol is first transformed into 3-hydroxypropionaldehyde and the final product for this route is 1, 3-propanediol (da Silva et al., 2009). Even though crude glycerol is released from the chemical reaction; the by-product could be directly used by microorganism to produce value added microbial products such as dihydroxyacetone (Liu et al., 2013), threonine rich edible fungus (Nitayavardhana and Khanal, 2011), and microbial omega 3 oil (Pyle et al., 2008).

Other biological wastes, such as waste from sago production, fruits peel, waste from newsprint, food waste, and waste from beer production, are also capable of being as substrates for microbial cultivation. Waste from sago liquid waste contains high content of complex carbohydrate (starch) which could be used by microorganisms (Yanti et al., 2017). Fruit waste such as banana peel could be hydrolyzed in order to obtain sugar and used as substrate for microbial cultivation (Preethi et al., 2017). Newsprint waste and waste from beer production could be consumed by microorganisms for producing bioethanol (Park et al., 2010; Ha et al., 2011). A fungus, *Aspergillus niger*, can consume food waste under submerge fermentation to produce an enzyme, glucoamylase (Wang et al., 2008).

As mentioned above, biological wastes or by-products still contain carbon source which can be consumed by microorganisms. The wastes or by-products can be transformed into value-added products by bioconversion processes of the microorganisms. AAB are the promising microorganisms that can be applied for the biological wastes or by-products bioconversion. For example, molasses and sugarcane bagasse could be used as substrate for *Acetobacter aceti* and *Komagataeibacter xylinus* cultivations for producing acetic acid and bacterial cellulose, respectively (Németh and Vidra, 2017; Cheng et al., 2017). In addition, crude

glycerol can be used as substrate for *Gluconobacter oxydans* cultivation to produce dihydroxyacetone (Zhou et al., 2016).

3. POTENTIAL AAB FOR VALUE-ADDED BIOLOGICAL WASTES

The biological wastes have potential as substrate for value-added microbial productions. Bioconversion of the wastes into other high-value products using AAB could be the reasonably practical method for the wastes management. AAB are obligately aerobic bacteria (Yamada et al., 1997), which are promising candidates for adding value of the biological wastes because the bacteria are capable of consuming various carbon sources including ethanol, glycerol, acetate, lactate, sugars (glucose, fructose, sucrose, etc.), and sugar alcohols (Mamlouk and Gullo, 2013). AAB have membrane bound dehydrogenase enzymes that can oxidize various carbon sources into other high value products (Yakushi and Matsushita, 2010). AAB have been obviously used for converting biological wastes including molasses, crude glycerol, bagasse, fruits peel, and other biological wastes to produce many high-value chemicals such as acetic acid (Patel and Pandya, 2015; Németh and Vidra, 2017; Preethi et al., 2017), dihydroxyacetone (Liu et al., 2013; Poljungreed and Boonyarattanakalin, 2017), gluconic acid (Sainz et al., 2016; García-García et al., 2017), and bacterial cellulose (Bae and Shoda, 2005; Cheng et al., 2017; Kasim and Rahman, 2016; Costa et al., 2017; Yanti et al., 2017). *Acetobacter* and *Gluconobacter* are the genera of AAB that have been used for increasing the value of biological wastes. Table 1 shows potential AAB that have been cultivated using biological wastes as substrate for producing other high-value products.

Acetobacter aceti

Acetobacter aceti are well known as the producer of acetic acid. Acetic acid is applied for the production of vinegar which is supplied in food market. Acetic acid could be produced by either chemical or biological process (Németh et al., 2017). However, in order to supply for food aspect, acetic acid is usually produced by biological cultivation of *Acetobacter aceti* using ethanol as a substrate. Acetic acid up to 164 g/L could be produced by conventional method of *Acetobacter aceti* cultivation using ethanol as substrate (Németh and Vidra, 2017).

Many biological wastes including Food waste, molasses, and fruits peel have been used as substrates for acetic acid production by the cultivation of *Acetobacter aceti*. Since *Acetobacter aceti* produces acetic acid using ethanol as a substrate. The biological wastes which mostly contain organic matters were pretreated using chemical process or biological processes before using as substrate for *Acetobacter aceti* cultivation. Fruits peel were heated and acid hydrolyzed before using as a carbon source for the acetic acid production (Preethi et al., 2017). Food waste and molasses were primary fermented by *Saccharomyces cerevisiae* in order to obtain ethanol which was further used as carbon source for *Acetobacter aceti* cultivation to produce acetic acid and up to 25 g/L (product-substrate yield coefficient (Yp/s) of 10.37% based on total solid of the substrate) of acetic acid was obtained (Li et al., 2015; Patel and Pandya, 2015).

According to the above details, metabolic reactions for acetic acid production using biological wastes could be shown as Figure 1. There are two steps for acetic acid production by AAB using biological wastes as substrate. First, glucose which is a carbon source contained in biological wastes is converted into ethanol by yeast fermentation. The ethanol is then converted into acetic acid by AAB.

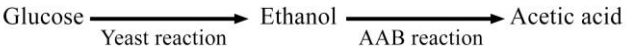

Figure 1 Metabolic reaction for acetic acid production by *Acetobacter aceti* cultivation using biological wastes as substrate.

Komagataeibacter xylinus

Komagataeibacter xylinus has been investigated for the ability to produce bacterial cellulose using biological wastes as carbon source. The bacterial cellulose is a high-value product which is applied as non-toxic biodegradable biopolymers (Mohammad et al., 2014).

Komagataeibacter xylinus could be found in acid fermentation such as wastes from vinegar fermentation (Aydin and Aksoy, 2009).

Komagataeibacter xylinus has been investigated for the production of bacterial cellulose using many biological wastes such as molasses, bagasse, and sago liquid waste. Molasses was pretreated with H_2SO_4-heat treatment in order to yield sugar before further use as a carbon source for *K. xylinus* cultivation (Bae et al., 2005). Acetic acid hydrolyzed bagasse was used as a low-cost carbon source for the production of valuable bacterial cellulose by *K. xylinus* (Cheng et al., 2017). Dry weight of bacterial cellulose with concentration of 4.12 g/L is obtained in *K. xylinus* cultivation using sago liquid waste as substrate (Yanti et al., 2017). The bacterial cellulose concentration is not significantly different from the conventional bacterial cultivation in Hestrin-Schramm medium (HS medium) which produced 4.15 g/L of bacterial cellulose (Möritz et al., 2013). Biosynthesis pathway of bacterial cellulose production by AAB is shown in Figure 2. According to Figure 2, AAB imports and converts glucose into glucose-6-phosphate (Glucose-6-P) and nucleoside diphosphate sugars (NDP-sugars), respectively. The NDP-sugars is then polymerized into exopolysaccharide (EPS) and exported outside (Freitas et al., 2011).

Gluconobacter oxydans

Besides *Acetobacter, Gluconobacter* is another promising genus of AAB which has potential on using biological wastes as substrate for high-value products generation.

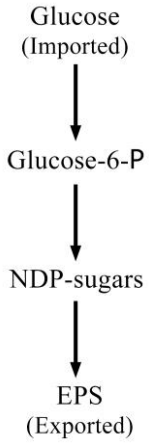

Figure 2 Exopolysaccharide production by AAB.

Gluconobacter oxydans are well-known as dihydroxyacetone producing bacteria. The acetic acid bacterium has been commercially used for the dihydroxyacetone production using glycerol as substrate and dihydroxyacetone up to 302 g/L could be produced by *G. oxydans* cultivation (Zhou et al., 2016). Biochemical reaction of dihydroxyacetone production from glycerol by AAB is shown in Figure 3. Glycerol is directly transformed into dihdroxyacetone by AAB using glycerol dehydrogenase (da Silva et al., 2009). Dihydroxyacetone is a very high-value chemical that could be applied in many industries including cosmetic, pharmaceutical, and foods (Hekmat et al., 2003). *G. oxydans* cultivation using crude glycerol as substrate could efficiently (Yp/s of 62%) generated dihydroxyacetone (Dikshit and Moholkar, 2016; Stasiak-Różańska et al., 2017).

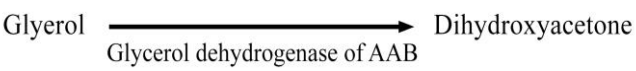

Figure 3 Dihydroxyacetone production by AAB using glycerol as substrate.

Another interesting application of *Gluconobacter oxydans* is its ability to produce up to 20 g/L of gluconic acid and xylonic acid using lignocellulosic hydrolysate

of wood chip as substrate (Lu, 2013). Gluconic acid and xylonic acid could be applied in food and pharmaceutical industries.

Gluconobacter frateurii

Gluconobacter frateurii has been recently investigated for dihydroxyacetone production using glycerol as a sole carbon source (Poljungreed and Boonyarattanakalin, 2017). *G. frateurii* cultivated in crude glycerol containing medium could practically produce dihydroxyacetone at high conversion yield (Liu et al., 2013; Zheng et al., 2016).

According to Table 1, *Acetobacter* and *Gluconobacter* are potential genera of AAB that have been used for adding value of biological wastes or by-products. *Gluconacetobacter hansenii* and *Gluconacetobacter diazotrophicus* are other two species of the AAB that possess the ability to convert low value biological wastes into high value products.

Gluconacetobacter hansenii

Gluconacetobacter hansenii presented ability to produce bacterial cellulose as a high-value product. *Gluconacetobacter hansenii* which was cultivated in alternative culture media containing glucose as a carbon source and corn steep liquor as a low-cost nitrogen source could produce bacterial cellulose at 73% of that achieved by the production using the conventional HS medium (Costa et al., 2017).

Gluconacetobacter diazotrophicus

Acetobacter diazophicus which was renamed as *Gluconacetobacter diazotrophicus* is another strain of AAB that has been studied for the production of acetic acid. *Gluconacetobacter diazotrophicus* which was isolated from sugar cane showed the production of indoleacetic acid (Fuentes-Ramirez et al., 1993). Indoleacetic acid is well-known as a plant hormone (auxin). *Gluconacetobacter diazotrophicus* was also known as a N_2 fixing bacteria which provided benefits for the growth of monocot plant (Eskin et al., 2014).

4. SUMMARY

AAB present promising applications in conversion of biological wastes or by-products into high-value products. *Acetobacter* and *Gluconobacter* are the two genera of AAB that could be applied for such bioconversion processes. *Acetobacter* and *Gluconobacter* could convert many low-cost biological wastes such as molasses, bagasse, food wastes, and crude glycerol into high-value products including acetic acid, bacterial cellulose, gluconic acid, and dihydroyxacetone. The bacteria are potential candidates that have feasibility for increasing value of biological wastes and consequently decreasing cost of industrial production processes.

Table 1 AAB used for converting biological wastes into high value products

Acetic acid bacteria	Biological wastes (Pretreatment methods)	Valuable products	References
Acetobacter aceti	Fruit peels (heated and acid hydrolysis)	Acetic acid	Preethi et al., 2017
Acetobacter aceti	Food waste from student cafeteria contains cooked rice, vegetables, meat and eggs (*Saccharomyces cerevisiae* fermentation)	Acetic acid	Li et al., 2015
Acetobacter aceti	Molasses (*Saccharomyces cerevisiae* fermentation)	Acetic acid	Patel and Pandya, 2015

Table 1 AAB used for converting biological wastes into high value products (Continued)

Acetic acid bacteria	Biological wastes (Pretreatment methods)	Valuable products	References
Komagataeibacter xylinus	bagasse (hydrolysate)	Bacterial Cellulose	Cheng et al., 2017
Komagataeibacter xylinus	Molasses (H_2SO_4-heat treatment)	Bacterial Cellulose	Bae et al., 2005
Komagataeibacter xylinus	Sago liquid waste	Bacterial Cellulose	Nur Arfa Yanti et al., 2017
Gluconobacter oxydans	Crude glycerol	Dihydroxyacetone	Dikshit and Moholkar, 2016
Gluconobacter oxydans	D-glucose	D-gluconic acid	Sainz et al., 2016
Gluconobacter frateurii	Crude glycerol	Dihydroxyacetone	Liu et al., 2013
Gluconacetobacter hansenii	Corn Steep Liquor	Bacterial Cellulose	Costa et al., 2017
Gluconacetobacter diazotrophicus	Sugar cane based isolation	Indoleacetic acid	Fuentes-Ramirez et al., 1993

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