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Review

Current Advances in the Production of 2,3-Butanediol by Microbial Fermentation

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Abstract

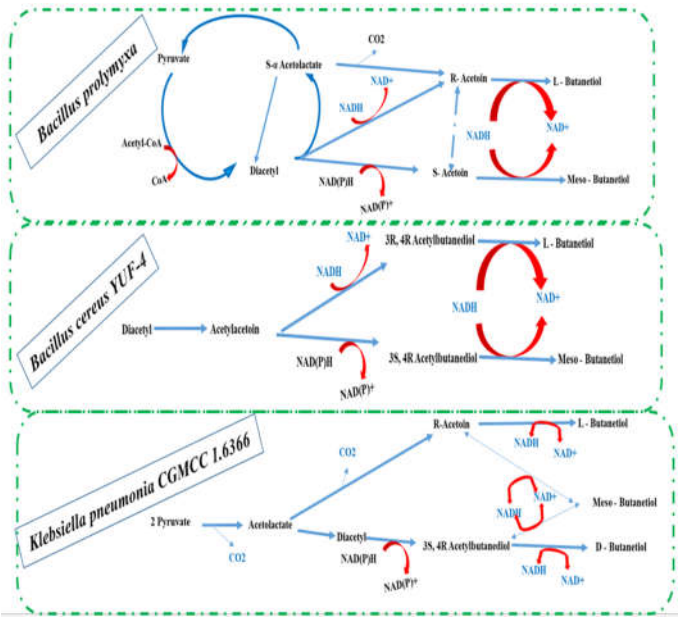
The 2,3-Butanediol (2,3-BD) is an extremely valuable bulk chemical attributable to an assortment of its applications. It can be produced by biotechnological routes from waste biomass, which makes it an alternative to traditional production. Due to expanding of energy interest, environmental concerns related towards the fossil fuels use and depletion, microbial fermentation can be an alternative for remediation of environment. The *Enterobacter* sp., *Bacillus polymyxa*, *Bacillus licheniformis*, *Pseudomonas* sp. and *Bacillus* sp. are gram-positive microorganisms, which are known as GRAS (Generally Regarded as Safe) bacteria. These are functional in 2,3-butanediol generation however, they have low productivity than *Klebsiella sp.* The potential that lies in genetic engineering and intensified fermentation methods combined with advancement methodology, for example, culture medium, pressure, temperature, pH and oxygen supply as well as making good bioreactors will stimulate rapid development of the process to improve of 2,3-BD. This review summarized the advances of microbial generation of 2,3-BD, metabolic regulation and metabolites of microorganisms for 2,3-butanediol production. Additionally, the challenges encountered in the production of microbial 2, 3-butanediol were also addressed in this review.

Keywords: 2,3-butanediol, metabolic engineering, microorganisms, down streaming, bioreactors.

Introduction

The 2,3-Butanediol (2,3 BD), as a butadiene precursor (Kim *et al.*, 2016) is broadly used in the rubber industry. It is a versatile chemical and can be used as a platform compound for industrial purposes. Additionally, 2,3 BD has potential applications in the manufacture of printing inks, perfumes, fumigants, moistening, softening agents, explosives, plasticizers, food. Also, it has a low freezing point of –60◦C which makes it a promising candidate for commercial use as an antifreeze agent (Rahman *et al.*, 2015; Priya *et al.*, 2016). The growing energy demand in environmental fossil fuels use and depletion with the inducements of biofuels production increased worldwide biodiesel production in the most recent years (Gallardo *et al.*, 2014). This process of biofuels production for microbial 2,3-butanediol consists of the enzymatic activity of 2,3-butanediol dehydrogenase (also known as acetoin/diacetyl reductase) can reduce diacetyl to acetoin and then to 2,3 BD (Fig. 1) that has three stereo-isomeric forms: meso-2, 3-butanediol, (2R,3R)-2,3-butanediol, and (2S,3S)-2,3-butanediol (Yu *et al.*, 2015). The focus on sugar fermentation for 2,3 BD production was reported in many studies and the high-effective productivities that have been succeeded through the conversion of glucose, however, the comparatively to high cost of conventional sugar substrates was still noticed as a major factor during 2,3-butanediol fermentation.

Fig. 1. Mechanisms of the formation of 2,3 BD stereoisomers (Sabra *et al.*, 2016).



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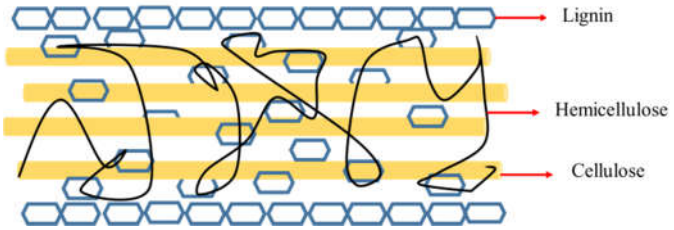
For that reason, 2,3-butanediol production using low-cost alternative way under suitable conditions is highly prioritized (Yang *et al.*, 2015b). Currently, the concern of microbial production of 2, 3 BD has been increased recently due to its industrial applications. A number of microbial species produce 2,3 BD by fermentation (Anvari and Safari, 2011) such as *Serratia marcescens, Paenibacillus polymyxa, Klebsiella oxytoca, K. pneumoniae* have been described to produce high yield on the other hand their pathogenicity was the major issue for industrial 2,3-butanediol production (Apoorva *et al.*, 2015). But *Enterobacter aerogenes , Bacillus polymyxa, Bacillus licheniformis* (Anvari and Safari, 2011), *Pseudomonas genus* (Jain, 1994) and *Bacillus subtilis* are gram-positive microorganisms that have been given GRAS status by the US Food and drug administration (Yang *et al.*, 2013a). This review summarized; previously understanding in biotechnological production of 2, 3-butanediol, sources of biomass used, employed microorganisms both wild type and genetically improved strains, as well as operating conditions applied. Additionally, the challenges may encounter in the production of microbial 2, 3-butanediol.

Microorganisms

Microorganisms able to ferment biomass to 2,3 BD has been isolated and described in order to achieve desired profitability. Many bacterial species can ferment pyruvate to 2,3 BD. In experimental process, the presence of the 2,3 BD pathway was identified in the following taxonomic groups: *Aeromonas hydrophila, Bacillus subtilis, Corynebacterium glutamicum, Enterbacter aerogenes, Klebsiella pneumoniae* (also known as *Aerobacter aerogenes*), *K. oxytoca, Lactobacillus brevis, L. casei, L. helveticus, L. plantarum, L. lactis, L. lactis subsp. lactis bv. diacetylactis, Leuconostoc lactis, L. mesenteroides subsp. cremoris, Oenococcus oeni, Pediococcus pentosaceus, Raoultella terrigena, Serratia marcescens, Bacillus polymyxa, Klebsiella terrigena, Streptococcus faecalis, Enterobacter cloacae, Bacillus licheniformis, B. amyloliquefaciens, Aerobacter indologenes, Pseudomonas chlororaphis* and the marine microalga, *Chlamydomonas perigranulata* (Celinska and Grajek, 2009). A number of microorganisms are able to amass 2, 3-BD, but few are known to produce significant quantities (Lu *et al.*, 2013). Bacteria and yeasts have been studied with the aim of producing 2,3 BD by microbial fermentation (Shrivastav *et al.*, 2013). The following species are mostly used to produce the diols like 2,3-butanediol; *K. pneumoniae, K. oxytoca, S. marcescens, Paenibacillus polymyxa,* and *Enterobacter aerogenes* (Zeng and Sabra, 2011). Among all these strains, *K. pneumoniae* and *B. polymyxa* have demonstrated their potential for industrial 2,3-butanediol production, especially, *K. pneumoniae* because of its wide-ranging substrate spectrum and cultural adaptability (Syu., 2001, Yang *et al.*, 2011). *The Enterobacter aerogenes* and *E. cloacae*, also can produce 2, 3-butanediol efficiently with high titer and productivity (Jullesson *et al.*, 2015).

Fig. 2. Lignocellulose structure showing cellulose, hemicellulose and lignin fractions (Teixeira, 2010).



The substrates

In the production of 2, 3-butanediol, the substrates are also the main components. The rapid accumulation of glycerol during biodiesel synthesis has attracted much interest on the potential usage. By using *Klebsiella pneumoniae*, glycerol is converted into 1,3-propanediol, as well 2,3-butanediol in low aeration (Yen *et al.*, 2014a). The final concentration of 2,3 BD reached 49.2 g/L after 280 h (Li *et al.*, 2013). Jatropha rich waste is an advantageous renewable feedstock for fermentation of sugars and 2, 3-butanediol production. Ionic liquid pretreatment and acid hydrolysis were considered for getting the great yield of water-soluble products and high concentration of reducing-sugars and benefited the fermentation of Jatropha hull hydrolysate to yield 66.58% diol with productivity improved from 0.35 up to 0.40 g/L/h (Jiang *et al.*, 2013). Lignocellulose in the form of forestry, agricultural, and agro-industrial waste is accumulated in large quantities every year (Fig. 2). These materials are mainly composed of three groups of polymers, namely cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are sugar rich fractions of interest for use in fermentation processes, since microorganisms may use the sugars for growth (Saddler, 1982) and production of value added compounds such as ethanol, food additives, organic acids, enzymes, and others (Teixeira, 2010). The major advantage of *Klebsiella sp*. has a wide-ranging substrate variety, including glucose, mannose, galactose, xylose, arabinose, cellobiose, and lactose. Hence, almost all of the sugars present in hemicellulose and cellulose hydrolysates can be converted to 2,3-butanediol. Without any treatment, the hydrolysate was successfully used to produce 2,3-butanediol and acetoin with a yield of 81.7% and a productivity of 0.7 g/L by *Paenibacillus polymyxa*. Higher concentration and higher productivity with relatively high yield, compared with previous works by acid hydrolysis, of 2,3-butanediol and acetoin were achieved (Jiang *et al.*, 2013). Sabra *et al.* (2015) reported different substrates, including glucose, xylose, Jerusalem artichoke powder, cassava powder, sucrose, inulin, and corn stover that have been used for 2,3-butanediol production.

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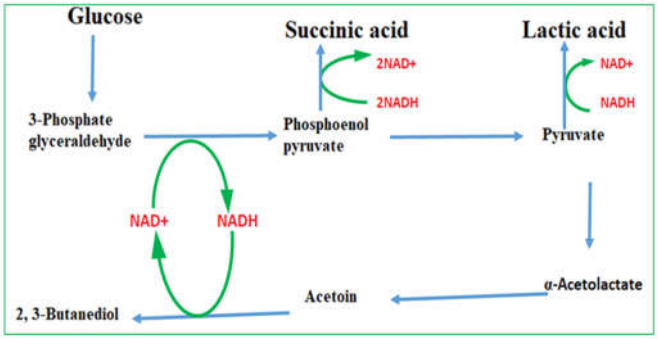
Fig. 3. Mixed acid-2, 3BD pathway (Yang *et al.*, 2013b).



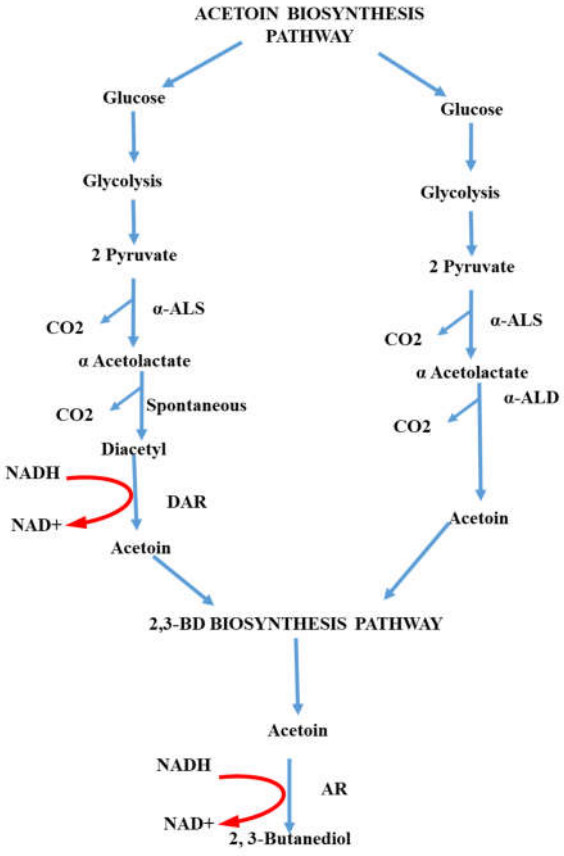
However, some microorganisms with a certain substrates indicated the by-products like acetoin (Sabra *et al.*, 2016) and mixed acid such as lactate, succinate and lactate (Fig. 3) (Yang *et al.*, 2013b). In the other hand, the metabolic engineering for 2,3-butanediol had reduced the accumulation of acetoin (Sabra *et al.*, 2016).

Metabolic pathway for the production of 2,3-butanediol

Depending on the microbial species, the 2,3-butanediol is produced from pyruvate in a mixed acid fermentation process (Volker *et al.*, 2011) via several intermediate compounds, including α-acetolactate, acetoin (acetyl methyl–carbinol), and diacetyl. The effect of succinic acid on the growth of *Enterobacter aerogenes* in the production of 2,3-butanediol was reported. Increasing succinic acid from 0 g/L to 30/L increased the final butanediol concentration. The maximum 2,3-butanediol productivity occurred at an initial succinic acid concentration was nearly 10 g/L (Anvari and Safari, 2011). Apart from 2,3-Butanediol some other end-products are synthesized, i.e. ethanol, acetate, lactate, formate and succinate, depending on the type of microorganism applied and their performing conditions (Fig. 3). Since most studies in this field have been carried out with members of the *Enterobacteriaceae* family, information presented here mainly concern this group of microorganisms. Primary, pyruvate from glycolysis can be converted either into lactate in a reaction which requires NADH (Johansen *et al.*, 1975) (catalyzed by L-/D-lactate dehydrogenase; LDH) or, after decarboxylation, into α-acetolactate (catalyzed by α-acetolactate synthase; α-ALS). α-acetolactate is mostly produced under low NADH availability. Furthermore, α-acetolactate can be converted into acetoin by α-acetolactate decarboxylase (α-ALD), and this reaction takes place under anaerobic conditions. If oxygen is present, α-acetolactate can undergo spontaneous decarboxylation producing diacetyl. Then, diacetyl reductase (DAR; also known as acetoin dehydrogenase) can convert diacetyl into acetoin. Finally, butanediol dehydrogenase (BDH; also known as acetoin reductase; AR) reduces acetoin to 2, 3BD (Voloch *et al.*, 1983).

It was reported, that acetate at low pH (i.e. acetic acid) is an effective inducer of all the three enzymes playing role in formation of 2,3 BD from pyruvate. All enzymes and compounds involved in 2,3 BD pathway are normally produced during the late log and stationary phases of fermentation, when oxygen-limiting conditions exist. The gene bdhA, coding for BDH, was recently identified and mapped in *Bacillus subtilis*. Which is particularly interesting that Nicholson suggested the existence of a second gene encoding a minor BDH/AR activity, since a very small amount of 2,3 BD was detected in bdhA-knock-out mutant. There is evidence, that in some cases one enzyme may carry both of the mentioned activities, e.g. L-BDH of *Brevibacterium saccharolyticum* catalyzes conversion of diacetyl to acetoin (with weak activity) and acetoin to 2,3 BD (Ui *et al.*, 2004). Another example is meso-BDH of *K. pneumoniae*, which interconverts acetoin and 2, 3 BD, but also has strong DAR activity (conversion of diacetyl to acetoin). In *Enterobacter aerogenes* also one enzyme carries the two activities. Hence, the new names diacetyl (acetoin) reductase or L-glycol dehydrogenase were proposed for the protein (Celinska and Grajek, 2009) (Fig. 4).

Fig. 4. Metabolic pathways of 2,3-butanediol production from glucose. Two alternative pathways of acetoin synthesis; α-ALS: α-acetolactate synthase; α-ALD: α-acetolactate decarboxylase; DAR: diacetyl reductase. AR: acetoin reductase (Celinska and Grajek, 2009; Maddox, 2008).



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Enhancing production of 2,3 butanediol through optimizing the influencing factors

The chemical composition of microorganisms can be quite varied depending upon such factors as the composition of the growth medium, the age of the culture, and the cell growth rate. All organisms contain the genetic information to produce a wide variety of enzymes and hence produce a great number of chemicals. However, only some enzymes are produced at all times, whereas others are greatly influenced by the substrate. Certain compounds interact with the substrate to repress the translation of genetic information for synthesis. (This process is called repression). Enhancement of product generation can be reached in a number of methods (Kent, 2003). Metabolic engineering has developed as a very powerful approach to optimizing industrial fermentation processes through the introduction of directed genetic changes using recombinant DNA technology. Successful metabolic engineering starts with a careful analysis of cellular function (Nielsen, 2001). Also Cultivation strategies are very important such as: Fed-batch cultivations (Li *et al.*, 2014; Priya *et al.*, 2016), batch cultivations, semi-continuous productions with immobilized cells, continuous cultivations and production with free cells, solid state fermentations. Therefore, it is also important to select the appropriate cultivation methods to use. The modulating fermentation parameters has an immense impact on the production yield of 2,3 BD. Many studies showed that microbial metabolism is affected by various oxygen content (Zeng *et al.*, 1990). Metabolism is also regulated by culture temperature, pH, and acetic acid supplementation. Genetic engineering has been employed to increase 2,3 BD production (Saddler, 1983).

Metabolic Engineering of Producing Strains

The 2,3-Butanediol (2,3 BD) is a valuable chemical that can be biosynthesized from many kinds of substrates. For commercial biological production of 2,3 BD, it is desirable to use cheap substrates (Zheng *et al.*, 2008; Chen *et al.*, 2013), this requires to improve the metabolic performance of microbes as efficient bio-factories for the production of the desired products (Jin *et al.*, 2014). The mutants can be selected depending the metabolic characteristics (Kent, 2003) for optimizing the metabolic pathways. The required new improved mutants can be obtained, by introducing a mutation (Zheng *et al.*, 2008) or altering some genes which are necessary in 2,3 BD pathway in order to overexpress the enzymes involved in 2,3 BD production process (Shin *et al.*, 2012a, Park *et al.*, 2015). The *K. pneumoniae* is the most powerful 2, 3BD producer which can utilize a wide range of substrates. However, many by-products are also produced by *K. pneumoniae*, such as ethanol, lactate, and acetate, which negatively regulate the 2,3 BD yield and increase the costs of downstream separation and purification (Zhang *et al.*, 2012).

Klebsiella pneumoniae mutants with lactate dehydrogenase (LDH), acetaldehyde dehydrogenase (ADH), and phosphotransacetylase (PTA) deletion individually has been constructed by suicide vector conjugation (Guo *et al.*, 2014). These mutants showed different behavior of production formation. Knockout of ldhA had little influence on the yield of 2,3 BD, whereas knockout of adhE or pta significantly improved the formation of 2,3 BD. The accumulation of the intermediate of 2,3 BD biosynthesis, acetoin, was decreased in all the mutants. The mutants were then tested in five different carbon sources and increased 2,3 BD was reported. Also a double mutant strain with deletion of adhE and ldhA was constructed which resulted in accelerated fermentation and higher 2,3 BD production. In fed-batch culture, this strain achieved more than 100 g/L 2,3 BD from glucose with a relatively high yield of 0.49 g/g by the inactivation of adhE and pta. The inactivation of ldhA could advance faster cell growth and shorter fermentation time. The double mutant strain with deletion of adhE and ldhA resulted in accelerated fermentation and higher 2,3 BD production (Guo *et al.*, 2014).

Corncob molasses was used to produce 2,3 BD, a waste by product in xylitol production, contains high concentrations of mixed sugars by using *Klebsiella pneumoniae SDM*. This indicated that *K. pneumoniae SDM* can utilize various sugars contained in the corncob molasses in a preferential manner: glucose > arabinose > xylose. However, high sugars concentration had an inhibitory effect on the cells growth and 2,3 BD production, the low-cost corncob molasses could be used as an alternative substrate for the production of 2,3 BD by *K. pneumoniae SDM*, as well as a potential carbon source for production of other high-value chemicals. The maximum concentration of 2,3 BD was 78.9 g/L after 61 h of fed-batch fermentation, giving a 2,3 BD productivity of 1.3 g/L h and a yield of 81.4% (Wang *et al.*, 2010). Therefore, the sugar concentrations, temperature, pH, agitation, various monosaccharides and multiple sugar mixtures affect the 2,3 BD production productivity, yield and byproduct formation (Bothast, 1999). Most powerful producers of 2,3-butanediol are pathogenic, but there are some GRAS microbes like *Bacillus subtilis*, this species has acetoin reductase (Acr) catalyzes the conversion of acetoin to 2,3 butanediol with concomitant oxidation of NADH to NAD+ but the intracellular 2,3 BD production leads to the quantities of rate-limiting factor(s) Acr and/or NADH. Metabolic engineering strategies were proposed to redistribute carbon flux to 2,3 BD by manipulating NADH levels. The disruption of NADH oxidase (YodC, encoded by yodC) by insertion of a formate dehydrogenase gene in *Bacillus subtilis* was more efficient for enhancing 2,3 BD production and decreasing acetoin formation, like in *Bacillus amyloliquefaciens* B10-127 (Yang *et al.*, 2015b) than the disruption of YodC.

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This was because the former resulted in the recombinant strain AFY in which an extra NADH regeneration system was introduced and NADH oxidase was disrupted simultaneously. On fermentation by strain AFY, the highest 2,3 BD concentration increased by 19.9% while the acetoin titer decreased by 71.9%, relative to the parental strain. However, the concentration of lactate, the main byproduct, increased by 47.2%. To further improve carbon flux and NADH to 2,3 BD, the pathway to lactate was blocked using the insertional mutation technique to disrupt the lactate dehydrogenase gene ldhA. The resultant engineered strain *B. subtilis AFYL* could efficiently convert glucose into 2,3 BD with little acetoin and lactate accumulation. Through increasing the availability of NADH and decreasing the concentration of unwanted byproducts, this work demonstrates an important strategy in the metabolic engineering of 2,3 BD production by integrative recombinant hosts (Yang *et al.*, 2015a). Another improvement of 2,3 BD was reported on the co-expression of acetoin reductase (Acr) with NADH regeneration in *Bacillus subtilis,* where formate dehydrogenase and glucose dehydrogenase for NADH regeneration co-expressed with acetoin reductase in *Bacillus subtilis 168*. The yield and productivity were reached up to 74.5 g/L of 2,3 BD with 9.3 g/L/h productivity *Bacillus subtilis168/pMA5-bdhA-HpaII-fdh* and 63.7 g/L of 2,3 BD was produced with 7.92 g/L/h productivity *by Bacillus subtilis 168/pMA5-bdhA-HpaII-gdh* by fed batch and 115.4g of 2,3-BD (Samuel *et al.*, 2017).

pH Optimization

The p^H of the culture medium is an important factor, influencing many biological processes associated with microbial growth, metabolism, and ions uptake (Ho *et al.*, 2014). It is most important in 2,3 BD production because for different microorganisms and substrates, the ability of bearing the osmotic pressure variation, thus pH strongly affects the distribution of the metabolites in 2,3 BD fermentation. pH is taken as a governing factor in microbial conversion processes (Petrov and Petrova, 2010). Optimum values of pH are experimentally determined for 2,3 butanediol production by *Enterobacter aerogenes* through three set of batch fermentations of synthetic glucose solutions. The optimum pH value of 6.0 is evidenced from batch runs at variable pH, whose results are also used to make reasonable hypotheses on the reaction controlling the metabolic pathway which leads to 2,3 butanediol (Perego *et al.*, 2000). By using an isolated indigenous *Klebsiella* sp. *Ana-WS5,* the batch with pH controlled at 7.0 had the highest total diol (PDO + BD) productivity of 0.86 g/L h and the highest PDO/BD of 7.67, as compared to a batch with pH controlled at 6.0. However, the batch without pH control could achieve a maximum total diol concentration of 48.1 ± 1.6 g/L and the highest yield of 86% (total diols produced/glycerol consumed) (Yen *et al.*, 2014b). The pH on the *Klebsiella oxytoca* producing 2,3 BD has the range values (pH 5.5 and 7.0) (Park *et al.*, 2013). *Bacillus licheniformis* produced 2,3 BD from glucose with an optimum yield of 47 g/100g glucose after 72 h of growth on a peptone/beef extract medium containing 2% (w/v) glucose at pH 6.0 and 37°C. This yield of 2,3-butanediol was higher than those previously reported for *Klebsiella oxytoca* (37g/100g glucose) and *Bacillus polymyxa* (24g/100 glucose) (Nilegaonkar *et al.*, 1992). The highest yield production of 2,3-butanediol (2,3-BD) from glucose, after the optimized of the culture conditions for a lactate dehydrogenase-deficient mutant of *Klebsiella pneumoniae* using response surface methodology at the optimum pH (5.6) resulting in a maximum level of 2,3 BD production of 148.8g/L and productivity of 2.48 g/L/h compared to the 2,3 BD that was also obtained with high concentration (76.24 g/L) and productivity (2.31 g/L/h) from the *K. pneumoniae* mutant strain using sugarcane molasses as a carbon source(Lee *et al.*, 2014).

Cultivation Strategies

The 2,3-Butanediol production can be improved by designing a decent cultivation strategy (Kopke *et al.*, 2011), which require the control of substrate concentrations and evaluation of the feeding strategies whether batch or fed-batch (Cheng, 2010). The batch processes usually produce low productivity and substrate inhibition can easily occur (Wong, 2014). For overcoming this problem, the constant addition of the substrate such as glucose or molasses at an appropriate rate during the cultivation via fed-batch operation is preferred (Guo *et al.*, 2014). It was shown that high sugars concentration had an inhibitory effect on the cells growth and BD production. From the fed-batch fermentation, the maximum concentration of BD was 78.9 g/L after 61 h with a BD productivity of 1.3 g/L.h and a yield of 81.4%. The study suggested that the low-cost corncob molasses could be used as an alternative substrate for the production of BD by *K. pneumoniae SDM*, as well as a potential carbon source for production of other high-value chemicals (Wang *et al.*, 2010). The double fed-batch approach (daily additions of sugars together with yeast extract) was used under aerobic conditions, up to 88 and 113 g of combined butanediol and acetyl methyl carbinol per liter could be obtained from the utilization of 190 g of D-xylose and 226 g of D-glucose per liter, respectively (Saddler, 1983). The batch with a low DO could achieve a much higher PDO/BD ratio than the high DO batch, with results of 9.9 and 0.2, respectively (Yen *et al.*, 2014a). Generally, the substrate concentration is very important and must be measured and monitored. Fermentation was performed numerous times with molasses in repeated batch culture with cell recovery. Such repeated batch fermentation, in addition to a high product yield, also showed a very high product concentration.

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For example, 118 g 2,3-butanediol·L^{-1} and 2.3 g acetoin L^{-1} were produced from 280 g·L^{-1} of high test molasses. The diol productivity in this fermentation amounted to 2.4g·L^{-1}·h^{-1} and can undoubtedly be further increased by increasing the cell concentration. Because the *Klebsiella* cultures ferment 2,3-butanediol at an extremely high rate once the sugar has been consumed, the culture was inhibited completely by the addition of 15 g ethanol·L^{-1} and switching off aeration (Afschar *et al.*, 1991). From the experiments, it has been proven that molasses generates much more 2,3 BD because its composition of different sugars.

Aeration and agitation control

The oxygen as the influencing factor is the most important variable in the 2,3 BD fermentation. Batch fermentative production of 2,3-butanediol by *Klebsiella oxytoca* was investigated using various oxygen supply methods through varying agitation speed (Oladimeji, 2011). Higher cell concentrations and greater 2,3-butanediol production were observed in aerobic cultures of *Klebsiella oxytoca* than with anaerobic cultures. The concentration of butanediol inhibitors like ethanol and lactic acid are partially suppressed by adequate aeration-agitation. However, excessive aeration-agitation may lead to the formation of acetoin and acetic acid at the expense of butanediol (Qureshi, 1989). The ability of *Klebsiella oxytoca NRRL-B199* to use either lactose or the mixture of glucose and galactose as substrate for the production of 2, 3-butanediol was studied in batch fermentations with different conditions of aeration and pH. The 2,3-butanediol was undetected, or present in minute concentration in the fermentation broths with lactose, while it was the main product from glucose + galactose with final concentrations of up to 18.8 g/L in media at pH 6.0. Under conditions optimal for 2, 3-butanediol synthesis, when aeration limited growth, the rate of biomass growth was more tightly related to the aeration rate in lactose medium than in glucose + galactose medium. These relations suggest that the growth rate is very low on lactose but still considerable on glucose + galactose when aeration rate tends toward zero. Similarly, the metabolism is more oxidative in the some medium, yielding mainly acetate as product (Champluvier *et al.*, 1989). For 2,3 BD fermentation, the speed of agitation is very important. Huang *et al.* (2009) reported that the agitation speeds between 200 rpm and 300 rpm favored 2,3 BD fermentation 89.9 gL^{-1} while lower or higher agitation speeds resulted in lower final 2,3 BD concentration. The suggested reason was the high accumulation of ethanol and acetoin as the main byproducts. However, the agitation at 300rpm had highest productivity (1.44 gL^{-1} h^{-1}) suggesting that high concentration, high yield and high productivity of 2,3 BD could not be achieved simultaneously by controlling a constant agitation speed throughout the whole culture process (Ji, 2009).

Optimization of the temperature

Most bacterial species can grow on the temperature ranged between 30-35°C. The *K. pneumoniae TR17* strain produced hydrogen within a wide range of temperature (30-50°C), initial pH (4.0-9.0) and crude glycerol concentration (20-100 g/L) with yeast extract as a favorable nitrogen source. In batch cultivation, the optimal conditions for hydrogen production were: cultivation temperature at 40°C, initial pH at 8.0, 20 g/L crude glycerol and 2 g/L yeast extract (Chookaew *et al.*, 2012). By switching from aerobic to microaerobic conditions in this temperature range, fermentative products instead of biomass would be obtained. Above the optimum temperature, cells and enzymes are altered rendering the metabolism and 2,3-butanediol minimal. The suboptimal also make the regulation and rate of metabolism fail. It was found that in cultures of *K. pneumoniae*, lowering temperature from 35°C to 30°C resulted in a substantial reduction in ethanol synthesis in favor of 2,3 BD formation. If ethanol formation is repressed by a slight glycerol excess and lowered temperature, there are practically no byproducts. Under applied conditions a temperature of 33°C appeared to be optimal for *K. pneumoniae*. Changes in temperature had little effect on 2,3 BD formation (Celinska, 2009).

2, 3-Butanediol Downstreaming Process

The 2,3-Butanediol is one of the bulk chemicals that exhibited a wide range of potential uses in cosmetics and transport fuels as well as being a precursor of synthetic rubbers. During the last few years, considerable efforts have been made to improve the production of 2,3 BD from fermentation. However, major difficulties still exist in downstream processing because 2,3-butanediol has a high boiling point and a high affinity for water. Chief methods for the recovery of 2,3-butanediol comprise steam stripping, pervaporation, and solvent extraction. After many studies, there is lack of single method that has proved to be simple and efficient, therefore, the improvements are chiefly needed with esteem to yield, purity and energy consumption in process (Xiu and Zeng, 2008). The aqueous two-phase system described in this study may have potential application in the extraction of 2,3-butanediol produced by industrial fermentation processes (Sun *et al.*, 2009). It is reported that the aqueous two phase systems composed of water-miscible solvents and salts could be used to extract 2,3-butanediol from a model solution (Fig. 5). They used t-butanol, tetrahydrofuran and 2-propanol as water-miscible solvents, and dipotassium hydrogen phosphate, tri-potassium phosphate, potassium carbonate, potassium fluoride and tri-potassium citrate as salts.

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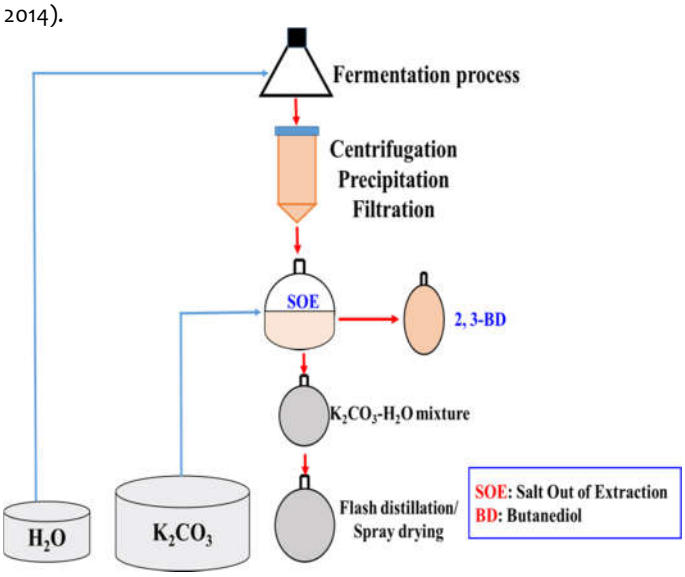
Fig. 5. Flow sheet of the downstream processing for 2,3 BD recovery from Pilot A Biorefinery process (Tim and Thorsten, 2014).



The phase separation abilities of water-miscible solvents and the salting-out abilities of salts were evaluated by the difference in the bimodal curves and salting-out of 2,3-butanediol. t-Butanol and tetrahydrofuran showed high phase separation abilities and potassium carbonate showed as high a salting-out ability as conventional phosphate salts (Matsumoto *et al.*, 2014). For economically, hybrid extraction-distillation (HED) was evaluated and indicated significant improved overall economics processes with oleyl alcohol that was found to be the most suitable solvent for the HED of 2,3 BD due to its high distribution coefficient and high selectivity (Harvianto *et al.*, 2018). Repulsive extraction or salting out using potassium chloride (KCl) or dehydrated K2CO3 was also investigated on the recovery of 2,3 BD 56 like the salting-out effect of K_2CO_3 on extraction of butanol in acetone–butanol–ethanol fermentation. The removal of water from the fermentation broth was also necessary before salting out because the concentration of 2,3-butanediol in the broth was too low to be salted out even if at a saturated KCl or K_2CO_3 solution (Tim and Thorsten, 2014).

Areas of 2, 3-butanediol applications and Perspectives

Currently, using biomass to produce chemicals and fuels, significantly, 2,3 BD is a potentially valuable fuel additive with a heating value of 27.2 kJ/g, comparable to that of other liquid fuels (e.g., ethanol 29.055 kJ/g and methanol 22.081 kJ/g). Because of the shortage of fossil fuels and the development of biorefineries from renewable resources, has received a significant amount of interest due to the approaching insufficiency of traditional fuels as well as the need for extra rational uses of food sources (Yang *et al.*, 2015a; Jiang *et al.*, 2015).

The 2,3-Butanediol is a multi-functional platform chemical that can be used to produce other bulk chemicals and synthesize diverse products, such as drugs, cosmetics, and industrial solvents and synthesis of polymers (Zeng and Sabra, 2011; Kim *et al.*, 2013). The 2, 3-Butanediol as an antecedent of butadiene, in addition to its applications in plastics, solvent, and antifreeze preparations (Shin *et al.*, 2012b). In the perspectives, an improved downstream processing of biologically produced diols, especially 2,3-butanediol should consider the time and economic basis of carbon sources and energy consumption in the whole process. Improving the conventional technologies and recent technologies for separation such as aqueous two-phase extraction with short chain alcohols, pervaporation, reverse osmosis, and in situ extractive or pervaporative fermentations deserve more attention in the future. Some bacterial are pathogenic even if they are 2,3-butanediol producing bacteria. Therefore, screening advancement of safe bacteria could be interest for biofuels development.

Conclusion

The2, 3-Butanediol is a very useful bulk chemical owing to a variety of its applications. It can be produced by biotechnological routes from waste biomass, which makes it an extremely attractive alternative to traditional production. The utilization of waste materials from renewable sources makes the process economically feasible and still, the bio-based synthesis needs to compete with less expensive chemical routes and in order to "win the battle" it needs improvement. One the problem in the microbial production of 2,3 BD is the low productivity and yield of the fermentation. Alternative solutions can be found especially by screening for safe microorganisms able to produce 2,3 BD. In this way, metabolic engineering of *Klebsiella* sp., naturally, they are pathogenic, has proven that it is possible to make hyper-producing and non-pathogenic strains. The *Enterobacter* sp., *Bacillus polymyxa, B. licheniformis*, the *Pseudomonas* sp., *Bacillus* sp. is a gram positive microorganism and it has been given GRAS (Generally Regarded as Safe) which are interesting in 2,3 butanediol production but they have low productivity than the *Klebsiella* sp. The potential that lies in genetic engineering and intensified fermentation methods coupled with optimization procedures such as culture medium, pH, pressure, temperature and oxygen supply as well as making worthy bioreactors will stimulate rapid development of the process to improve of 2,3 BD by eliminating byproducts synthesis especially ethanol, lactate and acetate. In short, the enhanced production of 2,3 BD relies on severe work, which includes genetic and metabolic engineering improved fermentation methods and parameters as well as the choice of decent and low cost substrates.

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References

1. Afschar, A.S., Bellgardt, K.H., Vaz Rossell, C.E., Czok, A. and Schaller, K. 1991. The production of 2,3-butanediol by fermentation of high test molasses. *Appl. Microbiol. Biotechnol.* 34(5): 582-585.
2. Anvari, M. and Safari, M.M.R. 2011. Enhancement of 2,3-butanediol production by *Klebsiella oxytoca* PTCC 1402. *Biomed. Biotechnol.* 2011 (2011): 7.
3. Apoorva, N.D., Sanjay, R.M., Tatyaso, B.Y., Dheeraj, M.M. and Rishi, J. 2015. Production of 2, 3-butanediol from sugarcane molasses using *Bacillus subtilis*. *Int. J. Adv. Biotechnol. Res.* 6(1): 66-79.
4. Bothast, B.C.S.R. J. 1999. Production of 2,3-butanediol by newly isolated *Enterobacter cloacae*. *Appl. Microbiol. Biotechnol.* 52(3): 321-326.
5. Celinska, E. and Grajek, W. 2009. Biotechnological production of 2,3-butanediol--current state and prospects. *Biotechnol. Adv.* 27(6):715-725.
6. Champluvier, B., Decallonne, J. and Rouxhet, P.G. 1989. Influence of sugar source (lactose, glucose, galactose) on 2,3-butanediol production by *Klebsiella oxytoca* NRRL-B199. *Arch. Microbiol.* 152(5): 411-414.
7. Chen, J.S., Zidwick, M.J. and Rogers, P. 2013. Organic acid and solvent production: butanol, acetone, and isopropanol; 1,3- and 1,2-propanediol production; and 2,3-butanediol production. *In:* Rosenberg, E., Delong, E.F., Lory, S., Stackebrandt, E. and Thompson, F. (eds.) *The Prokaryotes: Applied Bacteriology and Biotechnology*. Berlin, Heidelberg: Springer Berlin Heidelberg. pp. 77-134.
8. Cheng, K.K.L., Zhang, J.A., Li, J.P., Xu, J.M. and Wang, G.H. 2010. Improved 2,3-butanediol production from corncob acid hydrolysate by fed-batch fermentation using *Klebsiella oxytoca*. *Process Biochem.* 45(4): 613-616.
9. Chookaew, T., O-Thong, S. and Prasertsan, P. 2012. Fermentative production of hydrogen and soluble metabolites from crude glycerol of biodiesel plant by the newly isolated thermotolerant *Klebsiella pneumoniae* TR17. *Int. J. Hydrog. Energy*. 37(18): 13314-13322.
10. Gallardo, R., Faria, C., Rodrigues, L.R., Pereira, M.A. and Alves, M.M. 2014. Anaerobic granular sludge as a biocatalyst for 1,3-propanediol production from glycerol in continuous bioreactors. *Bioresour. Technol.*155: 28-33.
11. Guo, X., Cao, C., Wang, Y., Li, C., Wu, M., Chen, Y., Zhang, C., Pei, H. and Xiao, D. 2014. Effect of the inactivation of lactate dehydrogenase, ethanol dehydrogenase, and phosphotransacetylase on 2,3-butanediol production in *Klebsiella pneumoniae* strain. *Biotechnol. Biofuels.* 7(1): 44.
12. Harvianto, G.R., Haider, J., Hong, J., Van Duc Long, N., Shim, J.J., Cho, M.H., Kim, W.K. and Lee, M. 2018. Purification of 2,3-butanediol from fermentation broth: process development and techno-economic analysis. *Biotechnol. Biofuels.* 11:18.
13. Ho, S.H., Ye, X., Hasunuma, T., Chang, J.S. and Kondo, A. 2014. Perspectives on engineering strategies for improving biofuel production from microalgae--a critical review. *Biotechnol. Adv.* 32:1448-59.
14. Jain, S.K.G.A. 1994. Fermentative production of 2,3-butanediol: A review. *Bioresour. Technol.* 51(2-3): 103-109.
15. Ji, X.J., Du, J., Zhu, J.G., Ren, L.J., Hu, N. and Li, S. 2009. Enhanced 2,3-butanediol production by *Klebsiella oxytoca* using a two-stage agitation speed control strategy. *Bioresour. Technol.* 100(13): 3410-3414.
16. Jiang, L.Q., Fang, Z., Li, X.K. and Luo, J. 2013. Production of 2,3-butanediol from cellulose and Jatropha hulls after ionic liquid pretreatment and dilute-acid hydrolysis. *AMB Express.* 3: 48.
17. Jiang, L.Q., Zhen, F., Zeng-Li, Z., Fang, H. and Hai-Bin, L. 2015. 2,3-butanediol and acetoin production from enzymatic hydrolysate of ionic liquid-pretreated cellulose by *Paenibacillus polymyxa*. *Bioresour.* 10(1): 1318-1329.
18. Jin, H., Chen, L., Wang, J. and Zhang, W. 2014. Engineering biofuel tolerance in non-native producing microorganisms. *Biotechnol. Adv.* 32(2): 541-548
19. Johansen, L., Bryn, K. and Stormer, F.C. 1975. Physiological and biochemical role of the butanediol pathway in *Aerobacter (Enterobacter) aerogenes*. *J. Bacteriol.* 123(3): 1124-1130.
20. Jullesson, D., David, F., Pfleger, B. and Nielsen, J. 2015. Impact of synthetic biology and metabolic engineering on industrial production of fine chemicals. *Biotechnol. Adv.* 33(7):1395-402.
21. Kent, J.A. 2003. Industrial fermentation: Principles, processes, and products. *In:* Kent, J. A. (ed.) Riegel's handbook of industrial chemistry. Boston, MA: Springer US.
22. Kim, J.W., Kim, J., Seo, S.O., Kim, K.H., Jin, Y.S. and Seo, J.H. 2016. Enhanced production of 2,3-butanediol by engineered Saccharomyces cerevisiae through fine-tuning of pyruvate decarboxylase and NADH oxidase activities. *Biotechnol. Biofuels.* 9:265.
23. Kim, S.J., Seo, S. O., Jin, Y.S. and Seo, J.H. 2013. Production of 2,3-butanediol by engineered *Saccharomyces cerevisiae*. *Bioresour. Technol.* 146: 274-81.
24. Kopke, M., Mihalcea, C., Liew, F., Tizard, J.H., Ali, M.S., Conolly, J.J., Al-Sinawi, B. and Simpson, S.D. 2011. 2,3-butanediol production by acetogenic bacteria, an alternative route to chemical synthesis, using industrial waste gas. *Appl. Environ. Microbiol.* 77(15):5467-75.
25. Lee, S.M., Oh, B.R., Park, J.M., Yu, A., Heo, S.Y., Hong, W.K., Seo, J.W. and Kim, C. H. 2014. Optimized production of 2,3-butanediol by a lactate dehydrogenase-deficient mutant of Klebsiella pneumoniae. *Biotechnol. Biopro. Eng.* 18(6): 1210-1215.
26. Li, C., Lesnik, K. and Liu, H. 2013. Microbial conversion of waste glycerol from biodiesel production into value-added products. *Energies.* 6(9), 4739-4768.
27. Li, L., Chen, C., Li, K., Wang, Y., Gao, C., Ma, C. and Xu, P. 2014. Efficient simultaneous saccharification and fermentation of inulin to 2,3-butanediol by thermophilic *Bacillus licheniformis* ATCC 14580. *Appl. Environ. Microbiol.* 80(20):6458-64.
28. Lu, M., Park, C., Lee, S., Kim, B., Oh, M.K., Um, Y., Kim, J. and Lee, J. 2013. The regulation of 2,3-butanediol synthesis in *Klebsiella pneumoniae* as revealed by gene over-expressions and metabolic flux analysis. *Bioproc. Biosyst. Eng.* 37(3): 343-353.

***Corresponding author**

29. Maddox, I. 2008. Biotechnology Set. Institut für Mikrobiologie, Universität Münster, VCH Verlagsgesellschaft mbH. pp.269-291.
30. Matsumoto, M., Okuno, R. and Kondo, K. 2014. Extraction of 2,3-butanediol with aqueous two-phase systems formed by water-miscible organic solvents and inorganic salts. *Solvent Extr. Res. Dev.* 21(2):181-190.
31. Nielsen, J. 2001. Metabolic engineering. *Appl. Microbiol. Biotechnol.* 55(3):263-83.
32. Nilegaonkar, S., Bhosale, S. B., Kshirsagar, D. C. and Kapadi, A. H. 1992. Production of 2,3-butanediol from glucose by *Bacillus licheniformis*. *World J. Microbiol. Biotechnol.* 8(4) 378-381.
33. Oladimeji, A.T. 2011. Kinetics of Microbial production of 2, 3-butanediol from cheese whey using *Klebsiella pneumonia*. *Int. J. Biosci Biochem. Bioinforma.* 1(3).
34. Park, C., Lu, M., Yun, S., Park, K. and Lee, J. 2013. Effect of pH on the metabolic flux of *Klebsiella oxytoca* producing 2,3-butanediol in continuous cultures at different dilution rates. *Bioprocess Biosyst. Eng.* 36(6):845-55.
35. Park, J.M., Rathnasingh, C. and Song, H. 2015. Enhanced production of (R,R)-2,3-butanediol by metabolically engineered *Klebsiella oxytoca*. *J. Ind. Microbiol. Biotechnol.* 42(10): 1419-1425.
36. Perego, P., Converti, A., Del Borghi, A. and Canepa, P. 2000. 2,3-Butanediol production by *Enterobacter aerogenes*: selection of the optimal conditions and application to food industry residues. *Bioprocess Eng.* 23, 613-620.
37. Petrov, K. and Petrova, P. 2010. Enhanced production of 2,3-butanediol from glycerol by forced pH fluctuations. *Appl. Microbiol. Biotechnol.* 87(3):943-949
38. Priya, A., Dureja, P., Talukdar, P., Rathi, R., Lal, B. and Sarma, P.M. 2016. Microbial production of 2,3-butanediol through a two-stage pH and agitation strategy in 150l bioreactor. *Biochem. Eng. J.* 105: 159-167.
39. Qureshi, N.C. 1989. Effects of aeration on 2,3-butanediol production from glucose by *Klebsiella oxytoca*. *J. Ferment. Bioeng.* 67(6): 415-418.
40. Rahman, M., Shafiqur, Kesen, M. and Zhongshun, Y. 2015. Aerobic conversion of glycerol to 2,3-butanediol by a Novel *Klebsiella variicola* SRP3 Strain. *J. Microb. Biochem. Technol.* 7: 5.
41. Sabra, W., Groeger, C. and Zeng, A.P. 2016. Microbial cell factories for diol production. *Adv. Biochem. Eng. Biotechnol.* 155: 165-197.
42. Saddler, E. K. C. Y. a. J. 1983. Fed-batch approach to production of 2,3-butanediol by *Klebsiella pneumoniae* grown on high substrate concentrations. *Appl. Environ. Microbiol.* 46(3):630-635.
43. Yu, E.K. and Saddler, J.N. 1982. Enhanced production of 2,3-butanediol by *Klebsiella pneumoniae* grown on high sugar concentrations in the presence of acetic acid. *Appl. Environ. Microbiol.* 44(4):777-784.
44. Samuel, N., Bao, T., Zhang, X., Yang, T., Xu, M., Li, X., Komera, I., Philibert, T. and Rao, Z. 2017. Optimized whole cell biocatalyst from acetoin to 2,3-butanediol through coexpression of acetoin reductase with NADH regeneration systems in engineered *Bacillus subtilis*. *J. Chem. Technol. Biotechnol.* 92(9): 2477-2487.
45. Shin, H.D., Yoon, S. H., Wu, J., Rutter, C., Kim, S.W. and Chen, R.R. 2012a. High-yield production of meso-2,3-butanediol from cellodextrin by engineered *E. coli* biocatalysts. *Bioresour. Technol.* 118: 367-373.
46. Shin, S.H., Kim, S., Kim, J.Y., Lee, S., Um, Y., Oh, M.K., Kim, Y. R., Lee, J. and Yang, K. S. 2012b. Complete genome sequence of the 2,3-butanediol-producing *Klebsiella pneumoniae* strain KCTC 2242. *J. Bacteriol.* 194(10):2736-2737.
47. Shrivastav, A., Lee, J., Kim, H.Y. and Kim, Y.R. 2013. Recent insights in the removal of *Klebseilla* pathogenicity factors for the industrial production of 2,3-butanediol. *Microbiol. Biotechnol,* 23(7):885-896.
48. Sun, L.H., Jiang, B. and Xiu, Z.L. 2009. Aqueous two-phase extraction of 2,3-butanediol from fermentation broths by isopropanol/ammonium sulfate system. *Biotechnol. Lett.* 31(3):371-376.
49. Syu., M.J. 2001. Biological production of 2, 3-Butanediol. *Appl. Microbiol. Biotchnol.* 55(1): 10-18.
50. Mussatto, S.I. and Teixeira, J.A. 2010. Lignocellulose as raw material in fermentation processes. Current research, technology and education topics in applied microbiology and microbial biotechnology (Ed.). *Formatex*.pp. 897-907
51. Tim, F. and Thorsten, A. 2014. Design of downstream processing of non-gaseous products from finnoflag process (Report no: O 3.9. Institute for Biotechnology and Environmental Engineering. Wolfenbüttel, Germany). Retrieved from http://portal.savonia.fi/amk/sites/ default /files/pdf/eng/abowe/r.
52. Ui, S., Takusagawa, Y., Sato, T., Ohtsuki, T., Mimura, A., Ohkuma, M. and Kudo, T. 2004. Production of L-2,3-butanediol by a new pathway constructed in *Escherichia coli*. *Lett. Appl. Microbiol.* 39(6):533-537.
53. Volker, F., Steffen, N. and Tobias, M. 2011. Use of Glycerol in Biotechnological Applications. Biodiesel: Quality, emissions and by-products (Universidad Autónoma de Baja California, Mexico). pp. 305-341
54. Voloch, M., Ladisch, M.R., Rodwell, V.W. and Tsao, G.T. 1983. Reduction of acetoin to 2,3-butanediol in *Klebsiella pneumoniae*: a new model. *Biotechnol. Bioeng.* 25(1):173-183.
55. Wang, A., Wang, Y., Jiang, T., Li, L., Ma, C. and Xu, P. 2010. Production of 2,3-butanediol from corncob molasses, a waste by-product in xylitol production. *Appl. Microbiol. Biotechnol.* 87(3):965-970.
56. Wong, C.L. Y., H.W.; Lin, C.L.; Chang, J.S. 2014. Effects of pH and fermentation strategies on 2,3-butanediol production with an isolated *Klebsiella sp.* Zmd30 strain. *Bioresour. Technol.* 152: 169-176.
57. Xiu, Z.L. and Zeng, A.P. 2008. Present state and perspective of downstream processing of biologically produced 1,3-propanediol and 2,3-butanediol. *Appl. Microbiol. Biotechnol.* 78(6):917-926.
58. Yang, T.W., Rao, Z.M., Zhang, X., Xu, M.J., Xu, Z.H. and Yang, S.T. 2013a. Effects of corn steep liquor on production of 2,3-butanediol and acetoin by *Bacillus subtilis*. *Proc. Biochem.* 48(11): 1610-1617.
59. Yang, T., Rao, Z., Hu, G., Zhang, X., Liu, M., Dai, Y., Xu, M., Xu, Z. and Yang, S.T. 2015a. Metabolic engineering of *Bacillus subtilis* for redistributing the carbon flux to 2,3-butanediol by manipulating NADH levels. *Biotechnol. Biofuels,* 8(1):129.
60. Yang, T., Rao, Z., Zhang, X., Lin, Q., Xia, H., Xu, Z. and Yang, S. 2011. Production of 2,3-butanediol from glucose by GRAS microorganism *Bacillus amyloliquefaciens*. *Basic Microbiol.* 51(6):650-8.

***Corresponding author**

61. Yang, T., Rao, Z., Zhang, X., Xu, M., Xu, Z. and Yang, S. T. 2013b. Improved production of 2, 3-butanediol in *Bacillus amyloliquefaciens* by over-expression of glyceraldehyde-3-phosphate dehydrogenase and 2,3-butanediol dehydrogenase. *PLoS One,* 8(10):e76149
62. Yang, T., Rao, Z., Zhang, X., Xu, M., Xu, Z. and Yang, S.T. 2015b. Enhanced 2, 3-butanediol production from biodiesel-derived glycerol by engineering of cofactor regeneration and manipulating carbon flux in *Bacillus amyloliquefaciens*. *Microb. Cell Fact*. 14:122.
63. Yen, H.W., Li, F.T. and Chang, J. S. 2014a. The effects of dissolved oxygen level on the distribution of 1, 3-propanediol and 2, 3-butanediol produced from glycerol by an isolated indigenous *Klebsiella sp.* Ana-WS5. *Bioresour. Technol.* 153**:** 374-378.
64. Yen, H.W., Li, F.T., Wong, C.L. and Chang, J.S. 2014b. The pH effects on the distribution of 1, 3-propanediol and 2, 3-butanediol produced simultaneously by using an isolated indigenous *Klebsiella sp.* Ana-WS5. *Bioprocess Biosyst. Eng.* 37(3): 425-31.
65. Yu, M., Huang, M., Song, Q., Shao, J. and Ying, X. 2015. Characterization of a (2R,3R)-2,3-Butanediol Dehydrogenase from Rhodococcus erythropolis WZ010. *Molecules,* 20(4): 7156-7173.
66. Zeng, A.P., Biebl, H. and Deckwer, W.D. 1990. 2,3-Butanediol production by *Enterobacter aerogenes* in continuous culture: role of oxygen supply. *Appl. Microbiol. Biotechnol.* 33(3): 264-268.
67. Zeng, A.P. and Sabra, W. 2011. Microbial production of diols as platform chemicals: recent progresses. *Curr. Opin. Biotechnol.* 22(6):749-757.
68. Zhang, G., Yang, G., Wang, X., Guo, Q., Li, Y. and Li, J. 2012. Influence of blocking of 2,3-butanediol pathway on glycerol metabolism for 1, 3-propanediol production by *Klebsiella oxytoca*. *Appl. Biochem. Biotechnol.* 168(1):116-128.
69. Zheng, Y., Zhang, H., Zhao, L., Wei, L., Ma, X. and Wei, D. 2008. One-step production of 2,3-butanediol from starch by secretory over-expression of amylase in *Klebsiella pneumoniae*. *J. Chem. Technol. Biotechnol.* 83(10):1409-1412.

***Corresponding author**