45
2,3-Butanediol

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45.1 INTRODUCTION

Biomass conversion gives both pentoses and hexoses as products. While the hexoses (primarily glucose) are readily fermented, routes for pentose fermentation are still being developed. Hence, pentoses represent a potentially significant source of sugars with xylose being the major product.

Fermentation of xylose as well as glucose by ***Klebsiella oxytoca,*** **ATCC** 8724 (formerly known as ***Klebsiella pneumoniae*** and ***Aerobacter aerogenes)*** yields 2,3-butanediol as the major product. Other microorganisms capable of producing 2,3-butanediol (abbreviated 2,3-BD) include ***Bacillus subtilis*** (Ford strain), ***Aeromonas hydrophilia*** and several species of ***Serratia*** (Ledingham and Neish, 1954). Secondary products formed include acetoin, ethanol, lactic acid and glycerol. While ***K. oxytoca*** is able to yield high concentrations of 2,3-BD as mixtures of stereoisomers from monosaccharides, it is unable to utilize polysaccharides (Ledingham and Neish, 1954). In comparison, ***B. polymyxa*** is able to ferment starch directly giving L-2,3-butanediol and ethanol in almost equal amounts (Long and Patrick, 1963; Prescott and Dunn, 1959). However, ***B. polymyxa*** is unstable and difficult to maintain (Long and Patrick, 1963).

Both ***K. oxytoca*** and ***B. polymyxa*** have been used in pilot scale fermentation (Ledingham and Neish, 1954; Blackwood ***et*** *al.,* 1949), especially during World War II, as a possible means of producing 2,3-BD and subsequently 1,3-butadiene, an organic intermediate for rubber production. In the 1940s, process development was carried out through the pilot plant stage at the National

Research Laboratories in Ottawa, Canada. A 90% fermentation efficiency was attained on a 750-gallon scale for sugars obtained from whole wheat (Blackwood *et al.*, 1949). Process evaluation with barley as a feedstock indicated a 2,3-BD cost of 13 to 18 cents per pound (Tomkins *et al.*, 1948). Development was discontinued because less expensive routes for chemically producing **1,3-butadiene** from petroleum became available. In recent times, the long-term prospects of rising petroleum prices have revived significant interest in producing alcohols, including 2,3-BD, from biomass.

45.2 PROPERTIES

45.2.1 Stereochemical Configurations

There are three isomeric forms of 2,3-BD: D-(−), L-(+) and *meso* (Figure 1). Both the ***meso*** and (±) forms exist, to a considerable extent, in the conformation in which the hydroxy groups are ***gauche*** to each other (Figure 2). This conformation is favored because of the energy gained in the formation of the hydrogen bond. As a result, the methyl groups are ***gauche*** in the ***meso*** form and ***anti*** in the optically active form. Hence, the optically active form of the isomer is more stable than the ***meso*** form.

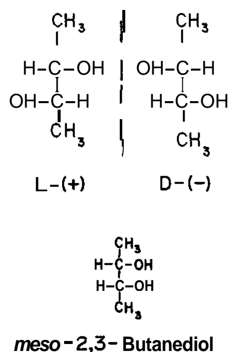

Figure 1 Three stereoisomers of 2,3-butanediol

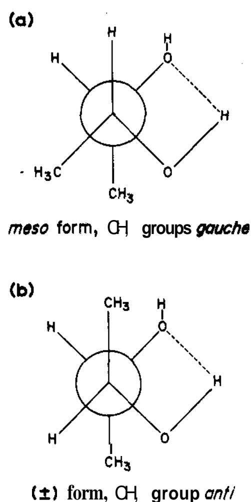

Figure 2 ***Anti*** **and** gauche forms of 2,3-butanediol

45.2.2 Physical Properties

Vapor–liquid equilibrium data for 2,3-butanediol are given by Othmer *et al.* (1945b) and other physical properties by Ledingham and Neish (1954). Briefly summarized, the water/butanediol equilibrium data show no azeotrope. The distillation of 2,3-butanediol removed the water overhead with 2,3-butanediol being the bottom product. While the boiling points of *meso* (181–182 "C), D (179–180 "C) and racemic (177 "C) 2,3-BD are slightly different, they all have boiling points much higher than water. Hence, the recovery of 2,3-BD from fermentation broth requires a large quantity of water to be evaporated. In practice, it was found that an excess of lime must be added to filtered fermentation liquor if 90% recovery were to be obtained through steam distillation. If the liquor was not filtered and lime was added, recovery was only 50% due to decomposition (Othmer *et al.*, 1945b). Process economics would dictate that essentially complete recovery be attained. An alternate approach suggested is liquid–liquid extraction, with *n*-butanol being a preferred extractant. Butanediol is then recovered as a bottom product in a subsequent distillation step with butanol being recycled (Othmer *et al.*, 1945a).

A major challenge in the economic production of 2,3-butanediol would still appear to be in separation of 2,3-BD from water (or fermentation broth) in an efficient manner. Hence, further research to carry forward the excellent work of these early pioneers seems needed.

45.3 FERMENTATION

45.3.1 Substrates

The single major cost in most biomass conversion processes appears to be the substrate cost (Ladisch *et al.*, 1983). Hence, the availability of an inexpensive carbohydrate material is essential for developing an economical fermentation process for production of 2,3-BD. Substrates suitable for the ***K. oxytoca*** fermentation include molasses (Long and Patrick, 1963), enzymatically hydrolyzed cereal mashes (Rose, 1961), acid hydrolyzed starch (Ward *et al.*, 1945) and wheat (Olson and Johnson, 1948), wood hydrolysates (Perlman, 1944) and sulfite waste liquor (Murphy and Stranks, 1951). ***B. polymyxa*** secretes amylolytic enzymes and hence is able to utilize cornstarch (Kooi *et al.*, 1948) and whole grain mashes of wheat (Blackwood *et al.*, 1949). The high post-fermentation solids content of whole grains impairs the recovery of 2,3-BD from the fermentation broth, and hence renders the use of such mashes to be less than optimum.

K. oxytoca and ***B. polymyxa*** are both able to utilize pentoses as well as hexoses (Ledingham and Neish, 1954). This is of considerable practical importance since hydrolysate from biomass materials can have pentose:glucose ratios of 1:1.5 (Tsao *et al.*, 1982). As a result, almost all of the sugar present in hemicellulose and cellulose hydrolysates can be converted to 2,3-BD (Flickinger and Tsao, 1979; Yu and Saddler, 1982).

45.3.2 Fermentation Conditions

The optimum pH for butanediol production by ***K. oxytoca*** is in the range pH 5.0–6.0 (Neish and Ledingham, 1949; Pirt and Callow, 1958; Jansen, 1984). The specific substrate utilization rate is maximum at pH 5.5 (Pirt and Callow, 1958). Above pH 6 the activity of one of the key enzymes in the butanediol pathway decreases sharply (Stormer, 1968).

The optimum temperature for growth, sugar uptake and butanediol production is 37 °C (Pirt and Callow, 1958; Esener *et al.*, 1981a). It is interesting to note that while 37 "C appears to be the best temperature for growth and fermentation, the highest butanediol concentration reported in the literature was achieved at 30 "C (Olson and Johnson, 1948).

The most important variable affecting the butanediol yield and the fermentation rate is the availability of oxygen. Although 2,3-BD is a product of anaerobic metabolism, aeration has been shown to enhance its production (Ledingham and Neish, 1954; Long and Patrick, 1963). Pirt and Callow (1959) suggested that aeration increases the butanediol productivity by increasing the cell concentration. However, too much aeration can decrease the yield of 2,3-BD. ***K. oxytoca*** is a facultative anaerobe which is able to obtain the energy it needs for growth by two different pathways: respiration and 'fermentation' (Figure 3). During oxygen limited growth (DOT < 5 mmHg as reported by Harrison and Loveless, 1971), both energy producing pathways are active simultaneously, and the yield of butanediol depends on the relative activities of each of the three pathways depicted in Figure 3. The butanediol yield can be maximized by minimizing the oxygen

availability because this limits respiration. However, with a small oxygen supply, little cell mass is produced and, therefore, the conversion rates are slow. The butanediol production rate can be maximized by increasing the oxygen supply rate because this leads to a higher cell density (Jansen, 1984).

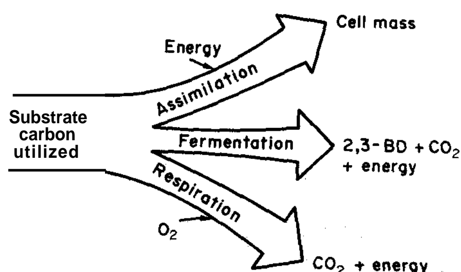

Figure 3 Pathways of substrate utilization for K. *oxytoca*

The oxygen supply rate is also important because the ratio of oxygen demand to oxygen supply can control the proportions of various metabolites produced (Vollbrecht, 1982). Fermentation products excreted by ***K. oxytoca,*** other than 2,3-BD, include acetoin, ethanol, acetate and others (Neish and Ledingham, 1949; Vollbrecht, 1982). In the absence of oxygen, ethanol is produced in approximately equimolar amounts with 2,3-BD (Neish and Ledingham, 1949). The presence of some oxygen appears to inhibit ethanol production. Increasing the oxygen supply rate toward the value of the potential oxygen demand results in an increase in the acetoin:butanediol ratio (Pirt and Callow, 1958). If the oxygen supply rate exceeds the microbial oxygen demand, oxygen is no longer limiting and the only products of sugar metabolism are cell mass and carbon dioxide (Pirt, 1957). Therefore, in order to maximize butanediol production, a limited but non-zero supply of oxygen is required.

Substrate concentrations used for this fermentation are generally in the range of 5–10% (Long and Patrick, 1963). Substrates commonly used in industrial-scale fermentations are usually diluted to even lower sugar concentrations. In experiments employing D-xylose as the sole carbon source, metabolic rates decreased significantly at xylose concentrations greater than 20 g l^{-1}. Indeed, when the xylose concentration exceeded 160 g l^{-1}, no growth at all was observed (Jansen, 1984). This apparent substrate inhibition may be explained by the dependence of the microbial growth rate on the water activity. When any solute decreases the water activity of the fermentation broth, the growth rate of ***Klebsiella*** also decreases (Esener ***et al.,*** 1981b). The low osmotic tolerance of K. ***oxytoca*** may be an important factor to consider in developing processes utilizing natural carbohydrate sources.

45.3.3 Fermentation Yields and Rates

The theoretical maximum yield of 2,3-butanediol from glucose is 0.50 g g^{-1}. The yield is the same from pentoses, which are converted to glyceraldehyde 3-phosphate by the pentose phosphate pathway (Jansen and Tsao, 1983). Actual butanediol yields obtained using ***K. oxytoca*** can exceed 80–90% of theory (Pirt and Callow, 1958).

The efficiency of the 2,3-butanediol fermentation can be judged by the product yield from sugar, the final butanediol concentration and the volumetric butanediol production rate. Table 1 lists values for these performance indexes that have been reported for various types of reactor configurations. These results were obtained using glucose or sucrose as the carbon source. When substrates of industrial interest are used, the butanediol yield is usually slightly lower (Blackwood ***et al.***, 1949; Tomkins ***et al.,*** 1948).

The fed-batch reactor has the capability of producing a high final butanediol concentration while minimizing the effects of initial substrate inhibition and final product inhibition. With continuous reactors, much higher rates are possible; however, product inhibition and incomplete substrate utilization are problems. The two-stage continuous culture system devised by Pirt and Callow (1959) is outstanding in its ability to rapidly produce a high butanediol concentration with a good yield from sugar. Another promising system employs immobilized cells in an attempt to

Table 1 Comparison of Yields for Different Types of Fermentation

Reactor type	Butanediol yield ($g\ g^{-1}$)	Butanediol concentration ($g\ l^{-1}$)	Butanediol productivity ($g\ l^{-1}\ h^{-1}$)	Ref.
Batch	0.43	65	1.6	Freeman and Morrison (1947)
Fed-batch	0.37	99	0.9	Olson and Johnson (1948)
Continuous	0.32	30	3.0	Pirt and Callow (1958)
Two-stage continuous	0.46	67	2.7	Pirt and Callow (1959)
Immobilized cells	0.25	3.4	1.3	Chua *et al.* (1980)

increase the conversion efficiency. The initial work of Chua *et al.* (1980) was carried out at pH 6.5 which may have caused the relatively poor performance. Future advances in the development of the immobilized cell reactor may result in a system that rivals or surpasses the two-stage continuous system in terms of overall performance.

45.4 BIOCHEMISTRY

The major intermediates in the conversion of a pentose or hexose to 2,3-butanediol are shown in Figure 4. The last step in the biological pathway of the fermentation involves the reduction of acetoin (2-hydroxy-2-butanone) to 2,3-butanediol. While 2,3-BD has two assymmetrical centers, acetoin has only one, and hence two stereoisomeric forms, D-(−) and L-(+). Hence, the reduction of acetoin to 2,3-BD may involve as many as two substrates and three products.

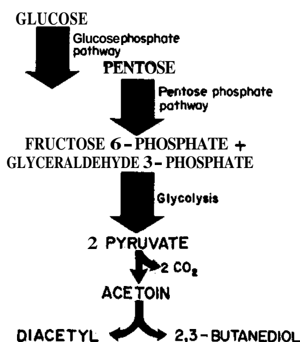

Figure 4 Major intermediates in conversion of a pentose or a hexose to 2,3-butanediol. Heavy arrows represent reactions of the pentose phosphate and glycolytic pathway. Light arrows indicate individual reactions

Juni (1952) reported that K. *oxytoca* forms acetoin from pyruvate by the action of two enzymes. An acetolactate-forming enzyme catalyzes the condensation of two pyruvate molecules combined with a single decarboxylation to yield acetolactate and CO_2. The decarboxylase is specific for the dextrorotatory isomer and the product is the levorotatory isomer of acetoin (D-(−)-acetoin). Both the decarboxylase and acetolactate-forming enzymes have been partially purified and characterized (Stormer, 1967; Stormer, 1968; Loken and Stormer, 1970; Malthe-Sorenssen and Stormer, 1970).

The acetoin can be oxidized to 2,3-butanedione (diacetyl) by O_2 present in the fermentation medium, or enzymatically reduced (with NADH as a cofactor) to 2,3-BD. Three stereoisomers are possible; indeed, for 40 years they have been known to exist in the fermentation broth. The isomeric composition varies with the microorganism used in the fermentation.

Walpole (1911) reported that K. *oxytoca* produced a mixture of *meso* and L-(+)-2,3-BD. *Aeromonas hydrophilia* and *Aerobacillus polymyxa* produce only D-(−)-2,3-BD (Stanier and Adams, 1944; Adams and Stanier, 1945; Neish, 1945). Bacterial oxidation of 2,3-BD is a function of the stereo configuration as summarized in Table 2 (Stanier and Fratkin, 1944; Sebek and Randles, 1952).

A mechanism for the formation of 2,3-BD stereoisomers by microorganisms was proposed by

Table 2 Bacterial Oxidation of 2,3-Butanediol by Various Microorganisms

Microorganism	*Observation*	*Ref.*
K. oxytoca	*meso* and L-(+) are oxidized	Stanier and Fratkin (1944)
A. polymyxa	D-(−) oxidized faster than *meso*	Stanier and Fratkin (1944)
A. hydrophila	Only *meso*-2,3-BD is oxidized	Stanier and Fratkin (1944)
Pseudomonas fluorescens	All three isomers of 2,3-BD oxidized	Sebek and Randles (1952)

Ledingham and Neish (1954). These investigators postulated the existence of two 2,3-BD dehydrogenases: (1) a dehydrogenase catalyzing reduction of D-(−)-acetoin to *meso*-2,3-BD, and (2) a dehydrogenase catalyzing reduction of D-(−)-acetoin to D-(−)-2,3-BD. The existence of an acetoin racemase was mentioned as a possibility. These investigators did not consider the formation of L-(+)-2,3-BD. Experimental data were not provided for the model.

In 1960, Taylor and Juni (1960) proposed a model for the formation of 2,3-BD stereoisomers from acetoin (Figure 5), based on the observed optical rotation of acetoin produced from pyruvate, the composition of 2,3-BD stereoisomers formed in the fermentation, and rates of oxidation of 2,3-BD stereoisomers. The model proposed the existence of three enzymes: an acetoin racemase, L-(+)-2,3-BD dehydrogenase and D-(−)-2,3-BD dehydrogenase. The dehydrogenases were said to be non-specific with respect to acetoin stereoisomers. That is, they would accept either acetoin isomer as substrate, but the reaction product would still be dependent on the acetoin isomer reduced. For example, the L-(+)-dehydrogenase would reduce L-(+)-acetoin to L-(+)-2,3-BD and D-(−)-acetoin to *meso*-2,3-BD.

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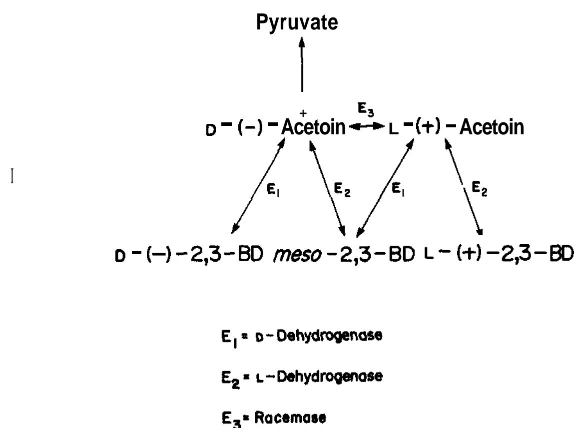


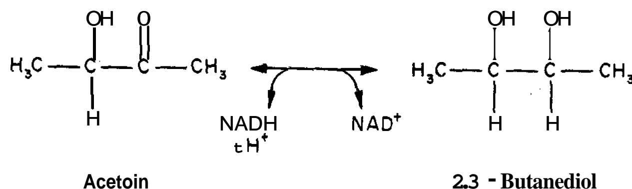
Figure 5 Mechanism for the formation of 2,3-butanediol stereoisomers by bacteria as proposed by Taylor and Juni (1960). For K. *oxytoca*, the presence of an acetoin racemase and L-(+)-2,3-butanediol dehydrogenase was proposed.

For *K.* oxytoca Taylor and Juni (1960) proposed the presence of acetoin racemase and the L-(+)-2,3-BD "dehydrogenase. While they were unable to document the presence of acetoin racemase in acetone-dried preparations of *K.* oxytoca, they speculated that cell extracts prepared by other procedures would be shown to contain an acetoin racemase activity.

45.4.1 Acetoin Reductase

Bryn et *al.* (1971) reported the purification and characterization of diacetyl (acetoin) reductase from *K.* oxytoca, which catalyzed the reduction of acetoin to 2,3-BD and the reduction of diacetyl to acetoin (Scheme 1).

Acetoin reductase is a tetramer with a molecular weight of 100 000 (Hetland et al., 1970). Upon isoelectric focusing of an apparently homogeneous preparation, at least 12 species all **pos**sessing enzymatic activity with respect to acetoin were detected (Hetland et al., 1971). Kinetic constants were determined using commercially available acetoin and 2,3-BD and are summarized in Table 3.



Scheme 1

Table 3 Kinetic Constants for Acetoin (Diacetyl) Reductase[a]

Constant	Value (μM)
K_m(NADH)	9
K_m(NAD^+)	180
K_m(acetoin)	530
K_m(2,3-BD)	11 300
K_i(NADH)	11
K_i(NAD^+)	140

[a] Phosphate buffer, pH 7.0, 35 "C (Larsen and Stormer, 1973).

The reduction of acetoin followed an ordered sequential Bi–Bi mechanism (Larsen and Stormer, 1973).

45.4.2 Kinetics of 2,3-Butanediol Formation from Acetoin

The results of Juni and co-workers and Ledingham and Neish (1954) have been extended by Voloch et al. (1983) to formulate a kinetic model based on studies using cell-free extracts from *K. oxytoca* grown on D-xylose, as well as acetoin reductases (E_1 and E_2) isolated from the extracts. The cell-free extract was found to have acetoin reductase activity. Based on their activities with respect to D-(−) and racemic acetoin, E_1 and E_2 were found to be *meso*-2,3-butanediol:NAD^+ oxidoreductase (D-(−)-acetoin forming) and L-(+)-2,3-butanediol:NAD^+ oxidoreductase (L-(+)-acetoin forming), respectively.

The kinetic model which describes the activities of these enzymes is shown in Figure 6. The major difference between this model and the one of Taylor and Juni (Figure 5) lies in the stereospecificity of acetoin reductases.

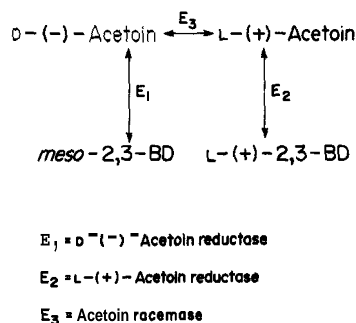


Figure 6 Modified mechanism for formation of 2,3-butanediol stereoisomers based on studies with enzyme activities isolated from K. *oxytoca* enzyme preparation

The properties and kinetics of D-(−)- and L-(+)-acetoin reductases have been determined by Voloch (1981). These reductases exhibit an ordered Bi–Bi mechanism.

The reaction equation for D-(−)-acetoin reductase is:

$$v = \frac{V_f V_r([A][B] - ([P][Q]/K_{app}))}{DEN} \tag{1}$$

where

$$DEN = V_r K_{iA} K_{mB} + V_r K_{mB}[A] + V_r K_{mA}[B] + \frac{V_f K_{mQ}[P]}{K_{app}} + \frac{V_f K_{mP}[Q]}{K_{app}} + V_r[B][A] + \frac{V_f K_{mQ}[A][P]}{K_{app} K_{iA}} + \frac{V_f [P][Q]}{K_{app}} + \frac{V_r K_{mA}[B][Q]}{K_{iQ}} + \frac{V_f K_{mB}[P][Q]}{K_{iB} K_{app}} + \frac{V_r [B][A][P]}{K_{iP}} \tag{2}$$

[A] = [NADH]
[B] = [D-(−)-acetoin]
[P] = [*meso*-2,3-BD]
[Q] = [NAD$^+$]
v = reaction velocity

$$K_{app} = \left(\frac{[P][Q]}{[A][B]}\right)_{eq}$$

The constants are defined in Table **4**.

In the absence of products, equation (1) reduces to:

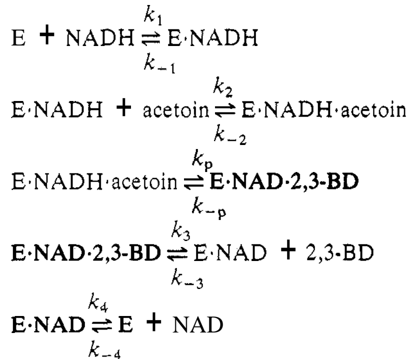
$$v = \frac{V_f [A][B]}{K_{iA} K_{mB} + K_{mB}[A] + K_{mA}[B] + [A][B]} \tag{3}$$

where v is the reaction velocity and the other parameters are defined in Table **4.** This equation represents the rate which would be expected during the initial period of reaction when product accumulation is small.

Table **4** Definition of Constants for the Ordered Bi–Bi Mechanism (Segal, **1975)**

Constant	Definition
K_{mA}	$k_3 k_4 / k_1 (k_3 + k_4)$
K_{mB}	$k_4 (k_{-2} + k_3) / k_2 (k_3 + k_4)$
K_{iA}	k_{-1} / k_1
K_{iB}	$(k_{-1} + k_{-2}) / k_2$
K_{mP}	$k_{-1} (k_{-2} + k_3) / k_{-3} (k_{-1} + k_{-2})$
K_{mQ}	$k_{-1} k_{-2} / k_{-4} (k_{-1} + k_{-2})$
K_{iP}	$(k_3 + k_4) / k_{-3}$
K_{iQ}	k_4 / k_{-4}
V_f	$k_3 k_4 [E]_t / (k_3 + k_4)$
V_r	$k_{-1} k_{-2} [E]_t / (k_{-1} + k_{-2})$

Constants refer to reaction sequence given in the general reaction sequences:



where the k's are kinetic constants, E_t = total enzyme, and stereo configurations given in Figure **5.**

Values of these constants based on initial rate studies for E_1 are given in Table **5.** The apparent equilibrium constant may be calculated by using a Haldane relationship (Segal, **1975):**

$$K_{app} = \frac{V_f K_{iQ} K_{mP}}{V_r K_{iA} K_{mB}} \tag{4}$$

Table 5 Kinetic Constants for Acetoin Reductases

Constant[a]	E_1 D-(−)-*Acetoin reductase value*	E_2 L-(+)-*Acetoin reductase value*
K_{mA}	7.4 μM	—
K_{mB}	460 μM	—
K_{iA}	10 μM	17 μM
K_{iB}	3900 μM	—
K_{mP}	2200 μM	—
K_{mQ}	56 μM	—
K_{iQ}	150 μM	20 μM
K_{iP}	29 000 μM	—
V_f	0.59 IU μg^{-1} protein	0.016 IU μg^{-1} protein
V_r	0.41 IU μg^{-1} protein	—
K_{iB}[b]	—	6600 μM

[a] A = NADH, B = D-(−)-acetoin, P = *meso*-2,3-BD, Q = NAD^+. 1 IU = formation of 1 μmol of product at 30 °C, pH 7.5. [b] B = L-(+)-acetoin.

Substituting the values for the constants given in Table 5 in equation (4) yields $K_{app} = 103$. The K_{app} determined experimentally has a similar value of 113. Hence, this shows that the kinetic constants obtained are consistent with the experimental data.

The data indicated that the enzymatic reduction of D-(−)-acetoin is essentially irreversible (i. e. $k_{-2} = k_{-p} = k_{-3} \approx 0$ in Table 4). A similar observation was made for L-(+)-acetoin. Hence, an integrated rate approach was used to determine the key kinetic constants for L-(+)-acetoin reductase. These values are also given in Table 5 (see E_2).

The magnitudes of the constants are similar to those reported by Larsen and Stormer in 1973 (see Table 3) although their model was different.

45.4.3 Analysis of Butanediol by Liquid Chromatography

Modeling of the reduction of the stereoisomers of acetoin to the stereoisomers of 2,3-BD requires an analytical tool which allows the separation of at least some of the stereoisomers. A liquid chromatography technique has been reported (Voloch *et al.*, 1981) which resolves *meso*- from L- and/or D-2,3-BD, from diacetyl or acetoin, and from their precursor sugar, xylose. This technique permits precise quantification without prior sample work-up and is suitable for preparation scale procedures.

The technique consists of injecting a 10 to 100 μl sample and eluting it with water through a 6 mm ID × 60 cm length column packed with Aminex 50W × 4 (Biorad Laboratories, Richmond, CA) at 85 °C. A typical chromatogram is shown in Figure 7. Details on the involved procedures for positively identifying the 2,3-butanediol peaks are given by Voloch *et al.* (1981).

45.5 CHEMISTRY

There are several interesting chemical reactions of 2,3-butanediol. These include dehydration to methyl ethyl ketone (industrial solvent), reaction with acetone to produce a 'tetramethyl' compound (a possible gasoline blending agent), and formation of butene and butadiene.

45.5.1 Preparation of Methyl Ethyl Ketone (MEK)

MEK can be produced by dehydration of 2,3-butanediol. The dehydration can be carried out using catalysts such as alumina or by direct reaction with sulfuric acid (Emerson *et al.*, 1982). The reaction mechanism involves a hydride shift (Scheme 2).

MEK is an industrial solvent and may find use as a liquid fuel additive.

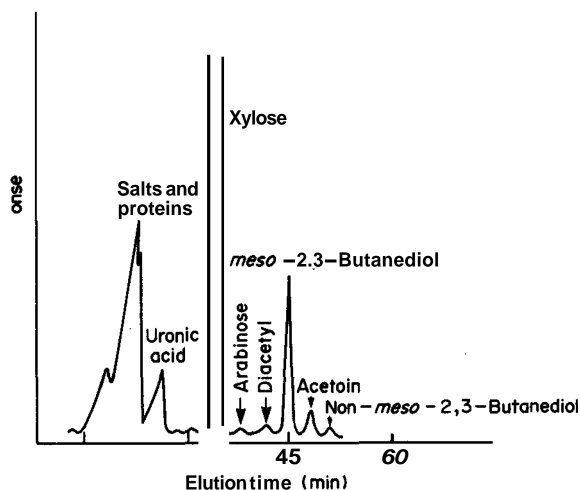
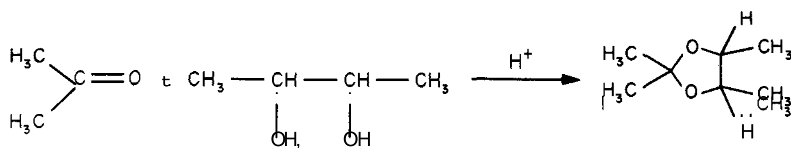


Figure 7 Liquid chromatogram of a 3 l fermentation broth of D-xylose by *K. oxytoca*, after 8 h. Fermentation conditions given by Jansen (1984)

Scheme 2

45.5.2 Tetramethyl Compound

The tetramethyl ether may find use as a blending agent for gasoline, similar to MTBE (methyl t-butyi ether). In fact, it has been pointed out that synthesis of MTBE and other new alkyl ether blending agents from sources other than petroleum stocks is essential if they are to be of much real benefit in extending gasoline supply (Stinson, 1979). Both acetone and 2,3-BD are fermentation products.

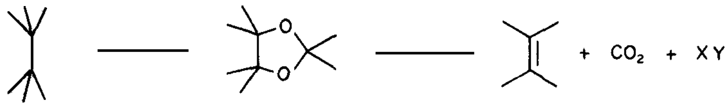


Scheme 3

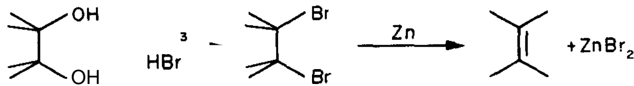
45.5.3 Preparation of 2-Butene and 1,3-Butadiene

Several reductive elimination reactions have been described in the literature (Corey and Winter, 1963,1965; Tipson and Cohen, 1965; Josan and Eastwood, 1968) which allow the conversion of a 1,2-diol into the corresponding alkene by the breakdown of the intermediate 1,3-dioxolane according to Scheme 4. These reactions proceed with a high degree of *syn* stereospecificity and can be readily applied to the preparation of cis and *trans* isomers of but-2-ene from 2,3-butanediol.

Treatment of the diol with PBr_3/HBr, followed by Zn powder, should also result in the formation of 2-butenes (Scheme 5). The debrominations also proceed with a high degree of *anti* stereospecificity (House and Ro, 1958; Gordon and Hay, 1968), the *meso* isomer giving the *trans*-butene, and the (+) isomer the *cis* butene.



Scheme 4

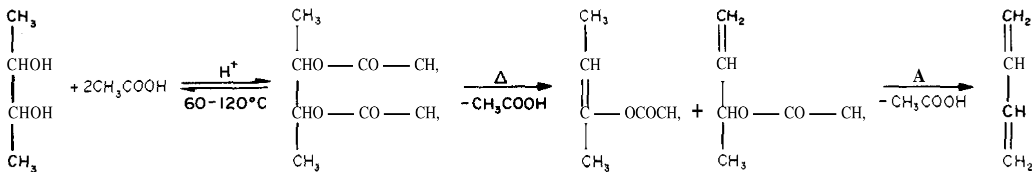


Scheme 5

The butenes can be catalytically dehydrogenated to 1,3-butadiene in the presence of superheated steam as a diluent and a heating medium (Kearby, 1955).

Butadiene can also be obtained in good yield by the direct dehydration of 2,3-butanediol over thoria catalyst, although most other dehydration catalysts give methyl ethyl ketone as the main product (Winfield, 1945).

Earlier work reported the esterification of 2,3-butanediol with acetic acid (Schlecter *et al.*, 1945b) followed by pyrolysis of the diacetate to butadiene (Scheme 6) (Schlecter *et al.*, 1945a).

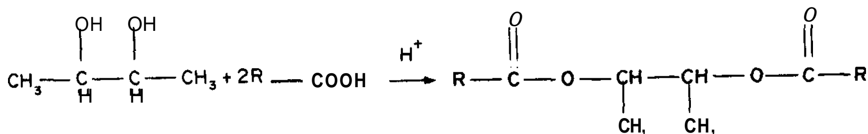


Scheme 6

Butene and butadiene are important industrial chemicals and are currently obtained from cracked petroleum.

45.5.4 Plasticizers

The esters of butanediol and suitable monobasic acids could find use as effective plasticizers for thermoplastic polymers such as cellulose nitrate, cellulose triacetate, cellulose acetate butyrate, polyvinyl chloride, polyvinyl esters, polyacrylates and polymethylacrylates. The diesters can be prepared by the usual esterification reactions with monobasic acids or their functional equivalents (Scheme 7).



Scheme 7

45.6 CONCLUSIONS

2,3-Butanediol is an example of a potential bulk chemical which can be produced by fermentation (Palsson *et al.*, 1981). While a process (Figures 8 and 9) for 2,3-BD recovery has been piloted (Wheat *et al.*, 1948), enhanced efficiency, both in energy consumption and product recovery, will aid the scale-up of the laboratory fermentations.

Figure 8 Pilot plant flowsheet (from Wheat *et al.*, 1948) for 2,3-BD recovery from fermentation beer

Meanwhile, further laboratory studies are warranted, for example, the development of strains of bacteria which are able to produce 2,3-BD from solutions containing high total solute concentration (derived from hydrolysis of biomass) at the same rate as from laboratory-prepared sugar solutions, and the development of more efficient bioreactors which will maximize conversion rate while minimizing the net production of cell mass. There is also need for further understanding of the kinetic behavior of the different enzymes involved in the biochemical pathway. The use of new analytical techniques has opened the way to re-evaluation of models existent in the literature.

The improvement of our knowledge of the 2,3-BD fermentation will not only enhance the chance of its commercial use but will also add to our general knowledge of bacterial fermentation.

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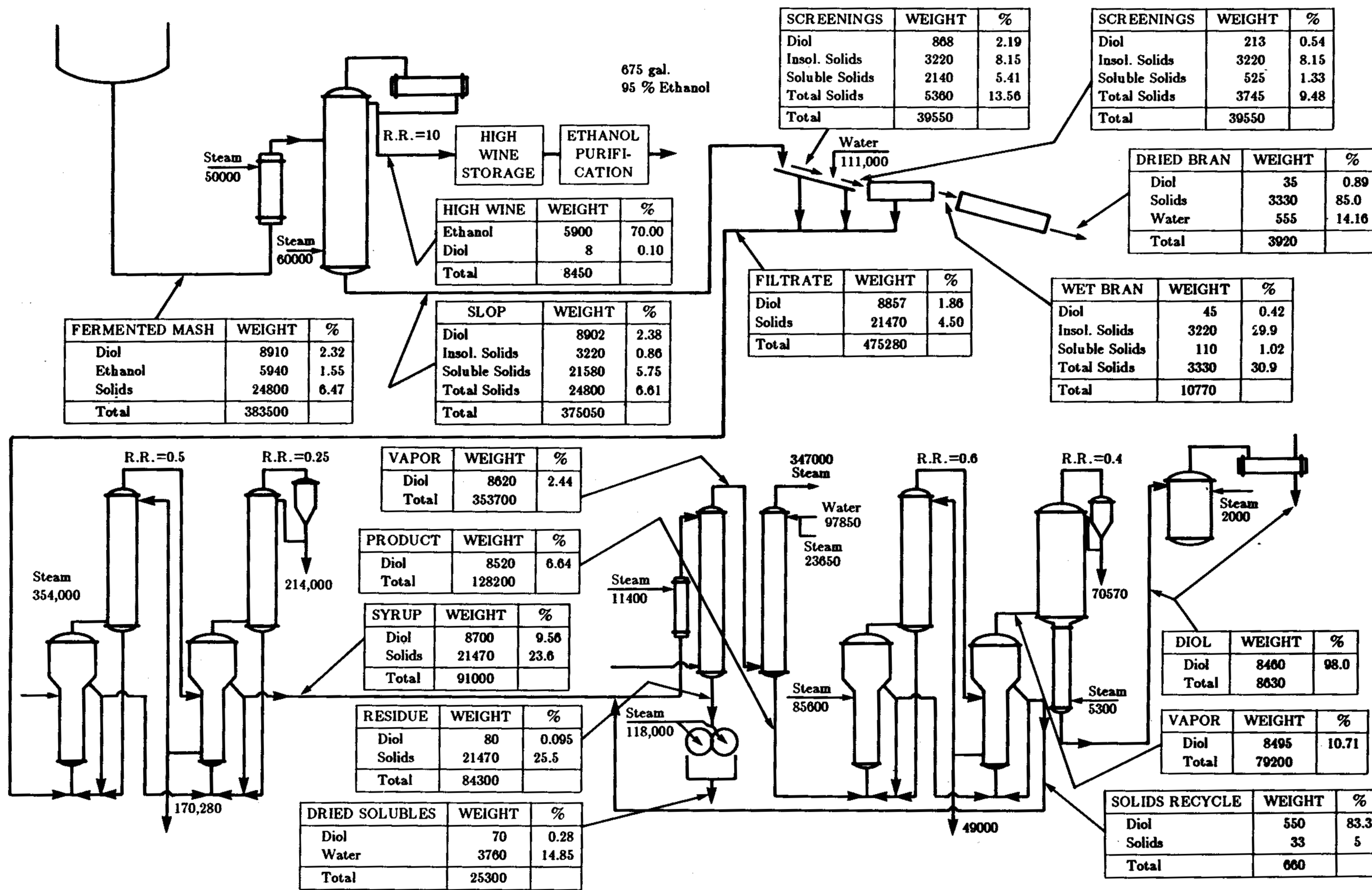

Figure 9 Material balance for commercial flowsheet for the production of (−)-2,3-BD based on 1000 bu. of wheat per day (from Wheat *et al.*, 1948)

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