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OXIDATION OF ALIPHATIC GLYCOLS BY ACETIC ACID BACTERIA

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INTRODUCTION

Many acetic acid bacteria are unsurpassed by other organisms in their ability to oxidize a great variety of carbohydrates and derivatives, including hexoses, pentoses, hexonic acids, primary and secondary alcohols, aldehydes, hydroxy acids, cyclitols, polyols, and aliphatic glycols. Most of these oxidations occur in one or two discrete steps, resulting in the accumulation of definite end products, often with a near-quantitative yield. Therefore, these bacteria are of considerable academic interest, and the reactions which

they stimulate are often of practical importance both in industry and in preparative organic chemistry.

Of particular interest are the oxidations of aliphatic glycols. These oxidations produce a series of interesting end products, which can be prepared easily and in good yield. Descriptions of these oxidations are scattered throughout the literature, and the reactions are poorly understood and, until recently (21), enzymatically unexplained. Some of these oxidations (e.g., of ethylene glycol and 2,3-butandediol) are commonly ascribed in textbooks as illustrations of the Rule of Bertrand-Hudson (2, 16*a*) for the oxidation of polyols; however, recent enzymatic work (21) has shown that they have no connection whatsoever with this rule.

It is the purpose of this review to discuss critically the available information on the oxidation of aliphatic glycols, with particular reference to the underlying enzymatic mechanism.

NOMENCLATURE OF ACETIC ACID BACTERIA

More than 50 different "species" names have been given to strains of acetic acid bacteria. This number was reduced to ten by Frateur (12), who also described a practical key to their identification. A "natural" classification and a further reduction of the number of species to two biotypes were proposed, and the biological implications were discussed, by De Ley (9). Nevertheless, in the older literature it is often quite difficult, and sometimes impossible, to ascertain the exact taxonomic position of the "species" used, in part because of an inadequate description and in part because misnomers are not infrequent. In the present review, we shall use the species names as mentioned by the authors in the original papers. For the sake of clarity, we summarize the most probable taxonomic position of various species in both Frateur's (12) and De Ley's (9) systems (Table 1).

CONDITIONS FOR GROWTH OF AND OXIDATION BY ACETIC ACID BACTERIA

Many of the older experiments on oxidations by acetic acid bacteria are of limited validity, because the optimal growth conditions of these bacteria were not realized. In these experiments, the bacteria were incubated in unshaken cultures, often with the substrate under investigation as the main carbon source. Under these conditions the bacteria grow very slowly and die quickly. In spite of the turbidity of the culture, most of the bacteria are dead after 1 to 2 weeks.

To carry out successful oxidation studies, the following requirements must be met.

(i) Inoculation of a growth medium has to be carried out with young cells, preferably 2, and not more than 3, days old.

(ii) Aeration has to be excessive; when submerged cultures are used, a rapid flow of finely dispersed sterile air is advisable.

TABLE 1. *Most probable taxonomic position of the strains mentioned in the present review**

Biotype according to De Ley (9)	Group according to Frateur (12)	Species according to other authors
Gluconobacter oxydans	suboxydans	*suboxydans*
		melanogenus
		oxydans
		industrium
Acetobacter aceti	mesoxydans	*aceti*
		xylinum
		?Termobacterium aceti
		?acetigenus
		liquefaciens
	oxydans	*pasteurianus*
		kuetzingianus
		rancens
		acetosus
		ascendens
	peroxydans	*paradoxus*
		peroxydans

* Doubtful positions are preceded by a question mark.

(iii) For growth, a rich culture medium is required, containing 1% yeast extract, tap water, and the substrate, and incubation at 25 to 30 C is recommended. The pH should remain between 4.5 and 6.5. Since many substrates are transformed into acids, finely divided $CaCO_3$ has to be added in somewhat larger amounts than those theoretically required.

(iv) Several substrates are oxidized quantitatively to definite end products, but do not serve as a carbon source. It is advisable to add a few per cent of glucose, glycerol, or ammonium lactate, which are good carbon sources for many strains. When glucose is used, $CaCO_3$ has to be added. When ammonium lactate is used, an indicator has to be incorporated in the medium

(e.g. bromocresol purple). The medium has to be acidified with concentrated HCl whenever it turns neutral.

(v) Several oxidizable compounds are inhibitory to growth. In this case, resting cells can be used with success. The bacteria are first grown for 2 days in a liquid or on a solid medium (containing, e.g., 5% glucose, 1% yeast extract, 3% $CaCO_3$, and tap water), and are then harvested, washed, and incubated at 30 C with the substrate under well-aerated conditions, e.g., on a shaking machine. It is necessary to adjust the pH occasionally.

If the above precautions are taken, a rapid (a few hours to a few days) and nearly quantitative conversion of many substrates (hexoses, pentoses, primary and secondary alcohols, glycols, polyols, etc.) will be obtained.

OXIDATION OF THE PRIMARY ALCOHOL FUNCTION

Ethylene Glycol

Ethylene glycol formed glycolic acid by the reaction:

$$\begin{matrix} CH_2OH \\ | \\ CH_2OH \end{matrix} \rightarrow \begin{matrix} COOH \\ | \\ CH_2OH \end{matrix}$$

Glycolic acid as an end product was identified in several ways: by the calcium content (4, 33, 42), crystal form (4), and crystal water (4) of the salt $(CH_2OH \cdot COO)_2Ca \cdot 2H_2O$; by the melting point of the free acid [78 C (18, 31), 73 to 75 C (21)]; as the phenylhydrazide [mp, 115 C (18, 31)]; as the *p*-bromophenacylester [mp, 137 to 138 C (21)]; and by paper chromatography (41).

Biological formation and preparation of glycolic acid. Brown in 1887 (4) was the first to show that *Bacterium aceti* oxidized glycol in a medium containing $CaCO_3$ and to isolate and identify calcium glycolate. This was subsequently confirmed by Seifert (33) with *Acetobacter pasteurianus* and *A. kuetzingianus*, and by Visser 't Hooft (42) with *Gluconobacter melanogenus*, *G. suboxydans*, *A. xylinum*, and *A. rancens*. Henneberg (17) also found acid formation from the same substrate. Although the end product was not identified, we can safely assume that it was only glycolic acid. A great variety of strains possess this ability: *A. xylinum*, *A. aceti*, *A. acetosus*, *A. kuetzingianus*, *A. pasteurianus*, *A. acetigenus*, *A. ascendens*, *G. oxydans*, *G. industrium*, and *Termobacterium aceti*.

Tanaka (35) reported that resting cells of *A. peroxydans*, *A. rancens*, and *A. aceti* oxidized ethylene glycol at 20, 3.5, and 4%, respectively, of the rate of ethanol oxidation. The oxidation was inhibited by HCN. According to Müller (30), *A. pasteurianus* killed by acetone treatment is still able to oxidize ethylene glycol with uptake of oxygen.

In the above experiments, the yield of glycolic acid, when mentioned, was very small, because the bacteria were grown in static cultures in which they lack oxygen for growth and oxidation. Seifert (33) reported only 8% glycolic acid with *A. pasteurianus* and 18% with *A. kuetzingianus*. Visser 't Hooft (42) reported 25% after 3 weeks of incubation with his strain (see above).

The yield can be considerably increased, however, by improved aeration. This was applied by Polesofsky (31) and Hromatka and Polesofsky (18) in submerged growth experiments. With an adapted culture in a medium containing inorganic salts, corn steep liquor, and 0.5% (v/v) ethylene glycol, the substrate was converted mainly during the logarithmic growth phase into glycolic acid with a nearly quantitative yield. Polesofsky (31) originally stated that no pure cultures were used; later Hromatka and Polesofsky (18) stated that a strain of *G. suboxydans* was involved. Submerged growth in a rich medium with plentiful aeration seems to be the method of choice for eventual industrial application. Small-scale laboratory preparation can easily be carried out with resting cells, previously prepared in large amounts, e.g., on 5% glucose, 1% yeast extract, 3% $CaCO_3$, as described by Kersters and De Ley (21).

Verloove (41) and Kersters and De Ley (21) studied the oxidation of ethylene glycol by resting cells of 15 strains, representing the entire taxonomic range. All strains oxidized the substrate only slowly and not beyond glycolic acid. *Gluconobacter* and *A. liquefaciens* are recommended for future work.

From the above results, it may be seen that the oxidation of ethylene glycol occurs with all strains of acetic acid bacteria. There is only one negative report in the literature with Bertrand's *A. xylinum* (2), which one is inclined to ascribe to a mishap.

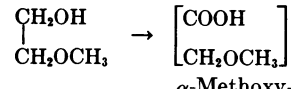
Intermediate formation of glycolic aldehyde. It can be expected that glycolic aldehyde might be an intermediate in the oxidation. Kaushal and Walker (20) trapped and characterized it as the 2,4-dinitrophenylosazone during growth of *A. acetigenus* in a synthetic medium with ethylene glycol as sole carbon source. This is the only report of the detection of an aldehyde as a result of the oxidation of the primary alcohol group of glycols by acetic acid bacteria. It is probable that an aldehyde is formed in each case, but oxidized at once. Waterman (43) found no reducing compounds in media with ethylene glycol, inoculated with *G. melanogenus*. Likewise, Polesofsky (31) and Hromatka and Polesofsky (18) were unable to detect glycolic aldehyde in submerged cultures with their strain of *G. suboxydans*.

Formation of oxalic acid. Banning (1*a*) reported that several species (*A. aceti*, *A. acetosus*, *A. ascendens*, *A. kuetzingianus*, and *A. pasteurianus*) formed abundant amounts of oxalic acid, whereas others (*A. acetigenus*, *A. xylinum*, *G. industrium*, and *G. oxydans*) did not. Calcium oxalate was identified by means of its crystal form, insolubility, formation of $CaSO_4$ with sulfuric acid, the crystal form of free oxalic acid, and the reaction of Kohl. However, we doubt the validity of these experiments, since Banning reported that he "sterilized" the medium (1% peptone, 1% meat extract, 1% substrate, 7% gelatine, and 1% agar) by heating at "etwa 75 C" and incubated, after inoculation, for about 30 days. Under these conditions, contamination seems unavoidable. Visser 't Hooft's observation (42) that *A. rancens* also forms oxalic acid has not been confirmed. Resting cells of a large variety of strains did not form oxalic acid (21, 41), nor did submerged cultures (18, 31). Furthermore, several authors reported that glycolic acid itself was not oxidized by a variety of strains of acetic acid bacteria (29, 34, 36, 37, 44). From these results, it can safely be deduced that acetic acid bacteria are unable to produce (or at most poorly capable of producing) oxalic acid from ethylene glycol.

Ethylene glycol as carbon source for growth. Waterman (43) found that this substrate supports vigorous growth of *G. melanogenus*. Mosel (29) cultured *A. ascendens* and *A. aceti* on 0.25% KH_2PO_4, 0.5% asparagine (as N source), and the substrate in stationary cultures. Slow growth was observed with both strains. According to Kaushal and Walker (20), *A. acetigenus* grows on a synthetic medium with ethylene glycol as sole carbon source, with the formation of a thick pellicle, most likely constituted of cellulose. The mechanism by which this C_2 substrate is converted into cell material is unknown.

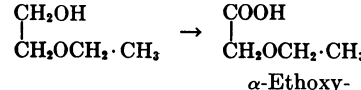
Derivatives of Ethylene Glycol

Ethylene glycol monomethylether (methylcellosolve). This compound was quickly oxidized by resting cells of *G. suboxydans* with the uptake of 1 mole of O_2 per mole of substrate, very likely according to the following reaction (21):



α-Methoxy-acetic acid

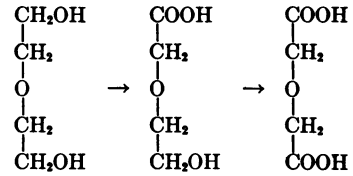
Ethylene glycol monoethylether (cellosolve). This compound was oxidized only very slowly and never to completion in submerged cultures of the *G. suboxydans* strain of Hromatka and Polesofsky (18, 31).



α-Ethoxy-acetic acid

The identity of the end product was shown by its boiling point (204 to 206 C) and as the piperazinium-bis-ethoxyacetate (mp, 122 C).

Diethylene glycol. In aerated submerged cultures of Hromatka and Polesofsky's strain of *G. suboxydans* (18, 31) the oxidation shown below was not always successful, and, when it happened, it was very slow and stopped before half of the substrate was converted.

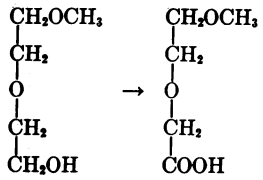


β-Hydroxy-ethoxy-acetic acid — Diglycolic acid

It is possible that the use of resting cells would improve the yield. β-Hydroxyethoxy-acetic acid was identified as its lactone 2-keto-1,4-dioxane (mp, 31 C). Diglycolic acid was identified by its

mp (148 C) and as the benzylthiuronium salt (mp, 154 C). The variable results and slow oxidation, as found by these authors, may explain why resting cells of one strain of *G. suboxydans* did not oxidize this substrate (7), whereas those of another did (21).

Diethylene glycol monomethylether. In submerged cultures of Hromatka and Polesofsky's strain of *G. suboxydans* (18, 31) the oxidation shown below went very slowly and did not reach completion.



2-(2-Methoxy)-ethoxy-acetic acid

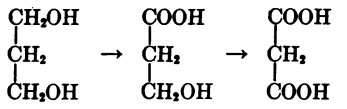
The yield was 36%. The end product was identified as the piperazinium derivative (mp, 43 C).

Triethylene glycol. This compound (CH_2OH–CH_2–O–CH_2–CH_2–O–CH_2–CH_2OH) was oxidized rapidly by resting cells of *G. suboxydans* with the uptake of 1 mole of O_2 per mole of substrate, probably by oxidation to an acid at one end of the molecule, followed by a slower oxidation at the other end (21).

Thiodiethylene glycol. This compound (CH_2OH–CH_2–S–CH_2–CH_2OH) was oxidized slowly by resting cells of *G. suboxydans* ATCC 621 (7). The end product(s) was not determined.

1,3-Propanediol (Trimethylene Glycol)

This compound was oxidized by the following reaction:



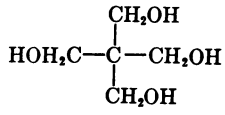
Hydracrylic acid (β-hydroxypropionic acid) — Malonic acid

Waterman (43) reported that *G. melanogenus* grew vigorously on yeast extract and 0.4% of the substrate. End products were not investigated. The *G. suboxydans* strain of Hromatka and Polesofksy (18, 31) oxidized this compound, but not to completion: the reaction stopped at 69 to 71% conversion. Hydracrylic acid was identified by the mp of its sodium salt (143 C) and as isoamylester (bp_{12} = 112 C). The second CH_2OH was also oxidized, although very slowly. The ratio of hydracrylic acid–malonic acid was about 22. The latter acid was characterized by its mp (133 C) and as benzylthiuronium salt (mp, 146 C).

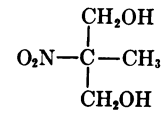
Resting cells of *G. suboxydans* likewise oxidized this compound to hydracrylic acid (21). The latter compound can easily be prepared by shaking the substrate with resting cells of, for example, *A. liquefaciens* (21). In this case, the end product was determined by the mp of its sodium salt (141 to 142 C) and by equivalent weight titration.

Derivatives of 1,3-Propanediol

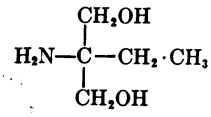
None of the compounds shown below was oxidized by resting cells of two different strains of *G. suboxydans:*



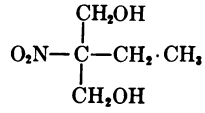
Penta-erythritol (7, 21)



2-Methyl-2-nitro-1,3-propanediol (7)



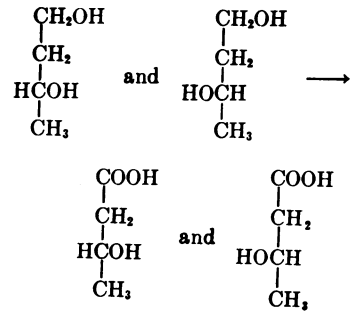
2-Ethyl-2-amino-1,3-propanediol (21)



2-Ethyl-2-nitro-1,3-propanediol (21)

D,L-1,3-Butanediol

All strains of acetic acid bacteria studied so far oxidized D,L-1,3-butanediol. *Gluconobacter* and the mesoxydans strains of *Acetobacter* oxidized it quickly to D,L-β-hydroxybutyric acid.



D,L-β-Hydroxybutyric acid

The oxidation rate with strains of the oxydans and peroxydans groups of *Acetobacter* depended on the strain used. Nevertheless, the end point of the O_2 uptake again indicated the formation of the same end products (21, 41). D,L-1,3-Butanediol is inhibitory for growth. The oxidation product could be prepared only with aerated resting cells. After 18 hr of shaking at 30 C, a yield of 70% was obtained, which probably could still be improved. The acid was isolated and characterized by mp of the sodium salt (163 to 164 C), as *p*-phenylphenacylester (mp 105 to 106 C) and by equivalent weight titration (21).

2-Butine-1,4-diol

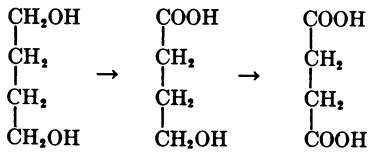
Cummins (7) reported no oxygen uptake with resting cells of *G. suboxydans* (ATCC 621). The *G. suboxydans* strain of Hromatka and Polesofsky (18, 31) did not oxidize 2-butine-1,4-diol ($CH_2OH \cdot C \equiv C \cdot CH_2OH$). It appeared to be toxic.

2-Butene-1,4-diol

Cummins (7) reported a slow O_2 uptake with *G. suboxydans*, exceeding 1.35 moles of O_2 per mole of 2-butene-1,4-diol ($CH_2OH \cdot CH = CH \cdot CH_2OH$). The end product was not identified, but might be fumaric acid.

1,4-Butanediol

Strains of *Gluconobacter* oxidized 1,4-butanediol to succinate (21, 41).



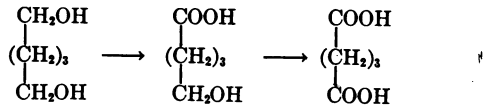
γ-Hydroxybutyric acid — Succinic acid

The reaction happens in two steps: (i) the uptake of 1 mole of O_2 per mole of substrate and formation of γ-hydroxybutyric acid, followed by (ii) the formation of succinic acid. The latter was easily prepared by shaking resting cells with the substrate and was identified by its mp (185 to 187 C) and as *p*-bromophenacylester (mp, 211 to 212 C). Nearly all the other strains of the mesoxydans, oxydans, and peroxydans groups of *Acetobacter* oxidized the substrate to near-completion. They, too, most likely form succinate but oxidize it subsequently by way of the Krebs cycle, whereas there is no indication that the Krebs cycle is operative in *Gluconobacter* (3, 22, 32).

Hromatka and Polesofsky (18, 31) studied this oxidation in submerged cultures with their strain of *G. suboxydans*. Highest acid production occurred with 0.4 to 0.75% (v/v) substrate. It corresponded to complete conversion into γ-hydroxybutyric acid; 0.96 and 1.5% substrate yielded only 70 to 90 and 60%, respectively, of the acid. The latter was isolated as its lactone, butyrolactone, and identified as γ-iodobutyric acid (mp, 40 C). This strain, under conditions of submerged growth, appears to accumulate mainly γ-hydroxybutyric acid and only a small amount of succinic acid (2 to 3%). The latter acid was identified by its mp (184 C) and as its bis-*p*-nitrobenzylester (mp, 88 C). It is surprising that both acids disappeared with this strain, apparently being converted to CO_2, because it has been reported that other strains of *Gluconobacter* lack the Krebs cycle (3, 22, 32). The results of Hromatka and Polesofsky tend to suggest that their strain might have a weakly active Krebs cycle, which escapes detection in the bacteriological identification tests; however, in prolonged fermentation with heavy aeration, low enzyme activity might still be responsible for a slow oxidation of succinate.

1,5-Pentanediol

Resting cells of our strain of *G. suboxydans* oxidized 1,5-pentanediol in two stages, each one with the oxygen uptake corresponding to the reactions shown below (21).

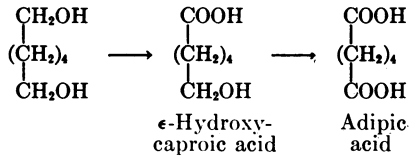


δ-Hydroxy-valeric acid — Glutaric acid

Glutaric acid was easily prepared by shaking resting cells with the substrate. It was characterized by its mp of 96 to 97 C and as the di-*p*-phenylphenacylester (mp, 151 to 152 C). Cummins (7) found slow oxidation with resting cells of his strain of *G. suboxydans*.

1,6-Hexanediol

1,6-Hexanediol was oxidized to adipic acid by the following reaction:



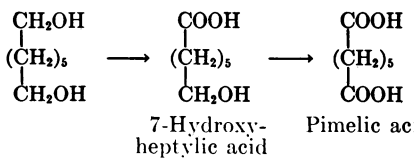
Resting cells of *G. suboxydans* oxidized this substrate rapidly to adipic acid, which was characterized by its mp (151 to 152 C) and as the *p*-bromophenacylester (mp, 153 to 154.5 C; 21). In submerged cultures of *G. suboxydans* (18, 31), 99% of the substrate was rapidly converted into adipic acid, which was determined by titration and identified by its mp (150 C) and as the *p*-bromophenacylester (mp, 158 C). The intermediate formation of ϵ-hydroxycaproic acid was established (identified as the hydrazide with a mp of 113 C). These authors also compared the oxidation rate of some ω-diols. 1,6-Hexanediol was oxidized fastest, followed by 1,4-butanediol, whereas 1,2-ethanediol was oxidized more slowly.

D,L-1,2,6-Hexanetriol

Our strain of *G. suboxydans* quickly oxidized this substrate ($CH_2OH \cdot CHOH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2OH$) with the uptake of 1 mole of O_2 per mole of substrate, possibly with the formation of 5,6-dihydroxy-caproic acid, followed by a slower oxidation, probably at the other end of the molecule (21). Cummins (7), too, found oxidation, albeit slow, with his strain of *G. suboxydans*. The end products have not yet been identified.

1,7-Heptanediol

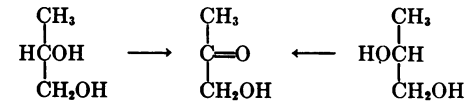
Resting cells of *G. suboxydans* oxidized this compound quantitatively into pimelic acid (21).



The intermediate formation of 7-hydroxyheptylic acid is likely, but this compound is probably oxidized as fast as 1,7-heptanediol. Pimelic acid can easily be prepared in this way, and was characterized by its mp (105 C) and as the *p*-phenylphenacylester (mp, 145 to 145.5 C).

OXIDATION OF THE SECONDARY ALCOHOL FUNCTION

D- and L-1,2-Propanediol



In this type of compound, with both a primary and a secondary alcohol function, it can not be predicted *a priori* which group will be oxidized. Experiments show that the mode of attack depends upon the distance between both groups in the molecule. When the groups are adjacent, as in 1,2-propanediol, only the secondary alcohol function is oxidized. When both groups are separated by a CH_2 group, as in 1,3-butanediol, the opposite occurs: the primary alcohol is oxidized, but the secondary CHOH is not attacked.

The interpretation of the older experiments on the oxidation of 1,2-propanediol is made difficult by the fact that only unshaken cultures were used, because it was not realized that acetic acid bacteria require an intense aeration. Kling (23), being primarily interested in the chemistry of keto alcohols, found the formation of a keto compound, which was subsequently identified as acetol (24). Kling used Bertrand's "bactérie du sorbose" (*A. xylinum*) and considered the oxidation of 1,2-propanediol as an application of the Rule of Bertrand, which had been proposed for the oxidation of sugar alcohols. This view has been reiterated in some handbooks. We know now (21) that this oxidation has no connection with the above rule (see below). Kling did not obtain more than 50% conversion, and the cultures became dextrorotatory. Kling rightly concluded that the (−) form is oxidized faster, which does not imply that the (+) form would not be oxidized at all. Kling (25) confirmed these results and extended the observations with other strains. Not all strains were effective, however, some being even totally unable to grow on this substrate. *Mycoderma aceti* (Orléans strain) acted like *A. xylinum*. In unshaken cultures, the oxidation stopped after 1 month. These results were confirmed by Visser 't Hooft (42) with *G. suboxydans*, *A. xylinum*, and *A. rancens*, also in unshaken cultures. The main end product again

was acetol. Small amounts of acetic acid and acetoin were also formed. The yield of acetol was calculated from the reducing properties: *G. suboxydans* produced 69.5% and *A. xylinum* 66%. Copet, Fierens-Snoeck, and Van Risseghem (6) used unshaken cultures. The racemic substrate was oxidized in a different way according to the strain used. These results may be summarized as follows:

$$\text{D}(-)\text{1,2-Propanediol} \xrightarrow[A.\ aceti]{A.\ xylinum} \text{acetol}$$

$$\text{L}(+)\text{1,2-Propanediol} \xrightarrow{G.\ suboxydans} \text{acetol}$$

With another strain of *G. suboxydans*, Van Risseghem (40) found quite the opposite, namely, that the (−) isomer was oxidized preferentially. However, these results have to be accepted with some reservations. First, no yields were given. It can be deduced from the results that the amounts of acetol were very small with *A. xylinum* and *A. aceti*, such that a configurational attack on this basis alone is open to doubt. Second, the cultures were preserved for a period up to 6 months (possibility for further oxidation of acetol).

When the results of the above authors are taken into account, it is tempting to conclude that with most strains the D(−) isomer is oxidized faster than the L(+) form. That *both* isomers are oxidized was shown by the much improved growth conditions of Butlin and Wince (5). A complete conversion into acetol was obtained with *G. suboxydans* under the following suitable conditions: (i) intense aeration, (ii) a pH between 4.5 and 6.5, and (iii) a suitable carbon source, such as glucose or glycerol. Quantitative conversion of the glycol in concentrations up to 15% can be obtained in less than 3 days. 1,2-Propanediol itself is a very poor carbon source, a fact which explains the limited conversion obtained by the previous authors.

The oxidation of the racemic mixture by resting cells of several strains was studied in our laboratory (21, 41). Strains of *A. aceti* and of *G. suboxydans* oxidized the substrate quantitatively to acetol. The same behavior of *G. suboxydans* ATCC 621 had been briefly reported by Cummins (7) and Goldschmidt and Krampitz (15). The oxidation rate with other strains of *Acetobacter* varied widely, from hardly any to very fast oxidation even beyond the acetol stage. Visser 't Hooft (42) had already observed that *A. rancens* produced acetol and consumed it afterwards. He proposed the following reactions:

$$\text{acetol} \rightarrow \text{pyruvate} \overset{\rightarrow CO_2}{\dashleftarrow} \text{acetaldehyde} \rightarrow \text{acetic acid}$$

$$\text{pyruvate} \downarrow \text{acetoin}$$

Acetol as the End Product of the Oxidation

Acetol as the result of bacterial oxidation was identified for the first time by Kling (24). It was characterized as the osazone (mp, 145 C), the hydrazone (mp, 97 to 98 C), and the oxime (mp, 70 to 71 C). Copet et al. (6) identified it as the semicarbazone (mp, 196 to 197 C). It is easily detectable with the Fehling reagent and as the 2,4-dinitrophenylhydrazone (21). Kling (26), in his review on the keto alcohols, mentioned that no aldehyde function was detectable with the fuchsine reagent. Huff (19) pointed out that the usual methods to identify acetol do not distinguish between this compound and lactaldehyde $CHO \cdot CHOH \cdot CH_3$. For instance, Kling identified acetol as its phenylosazone. This test does not distinguish between acetol, lactaldehyde, and several other compounds. Butlin and Wince (5) distilled the acetol in vacuo and assumed the compound to be acetol on the basis of the boiling point. However, it is known that, at atmospheric pressure, distillation of lactaldehyde yields acetol. Huff's criticism on the nature of acetol as an end product with acetic acid bacteria is invalidated by the following considerations. Copet et al. (6) crystallized the acetol-semicarbazone, with the same mp as Huff's compound, whereas lactaldehyde would not yield a precipitate under these conditions (19). Furthermore, Kersters and De Ley (21) found that the oxidation product from L(+)-1,2-propanediol was optically inactive, as expected for acetol; lactaldehyde would have been optically active. Thus, it seems more than plausible that acetol is indeed formed.

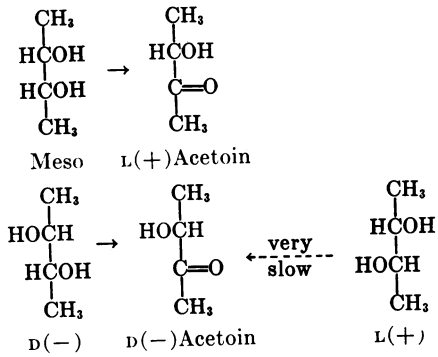
Derivatives of 1,2-Propanediol

Dipropyleneglycol ($CH_3 \cdot CHOH \cdot CH_2 \cdot O \cdot CH_2 CHOH \cdot CH_3$) and 2,5-dimethylhexine-3-diol-2,5 [$(CH_3)_2 \cdot COH \cdot C{\equiv}C \cdot COH \cdot (CH_3)_2$] are not oxidized by resting cells of *G. suboxydans* (7).

2,3-Butanediols

For the properties of the butanediols the paper of Ledingham and Neish (28) should be consulted.

The oxidation of meso-2,3-butanediol is often quoted as an illustration of the Rule of Bertrand. According to this rule, only the meso form could be oxidized, since it has two adjacent OH groups in the *cis* position. However, experience has shown that both the meso and D(−) forms are readily oxidized, whereas the L(+) form is slowly oxidized only at times.



Oxidation of racemic 2,3-butanediol. Kling (26) used 2,3-butanediol which had been prepared by reduction of racemic acetoin. He thus had the D,L mixture. Both the "bactérie du sorbose" (*A. xylinum*) and *M. aceti* oxidized the latter mixture in growing cultures. The residual glycol was dextrorotatory with $[\alpha]_D = 2°$. These results show that the D(−) form is preferentially oxidized over the (+) form. Kling's finding of 50% conversion is no proof that only one isomer was oxidized; it might have been a coincidence caused by poor aeration. Acetoin was detected as the semicarbazone (mp, 185 to 186 C) and as the osazone (mp, 242 C).

The first confirmation of Kling's results was obtained in the extensive and thorough investigations of Grivsky (16). *M. aceti* attacked only the D(−) diol. *A. xylinum* also attacked the same isomer first, but, because the optical rotation of the end product was low, Grivsky supposed that the L(+) form was also slowly oxidized. Underkofler et al. (38) again found that growing cultures of *G. suboxydans* oxidized the D(−) diol almost quantitatively to acetoin. The preparation of the L(+) compound was impure. Although oxidation occurred, the authors ascribed it to the presence of the meso form. However, it cannot be excluded that in this case the L(+) isomer was indeed oxidized. That some strains of *G. suboxydans* can indeed oxidize both D and L forms of 2,3-butanediol was shown by Kersters and De Ley (21). In conclusion, it can be said that all strains investigated oxidize the D(−) form, whereas the oxidation of the L(+) form is a matter of strain individuality.

Oxidation of meso-2,3-butanediol. Visser 't Hooft used 2,3-butanediol, slightly dextrorotatory, prepared with *Aerobacter aerogenes* from glucose. He considered this compound to be the D,L mixture with an excess of the L form, because of the sharp boiling point at 180 to 181 C. This interpretation is obviously incorrect, and this author almost certainly worked with the meso form, containing some of the L(+) compound, as may be seen from the following considerations.

(i) It is known now that *A. aerogenes* produces a mixture of about 90% meso- and 10% L(+)-2,3-butanediol. This is now indeed the usual method for obtaining the meso form.

(ii) The boiling point of meso is 181 to 182 C, of D(−) it is 179 to 180 C, and of D,L it is 177 C.

(iii) Visser 't Hooft reported that the anhydrous glycol solidified as a crystalline mass, obviously at room temperature. Racemic 2,3-butanediol, however, is liquid at this temperature (mp, 7.6 C), whereas the meso-form is solid (mp. 34.4 C).

We can thus disregard Visser 't Hooft's conclusions and concern ourselves only with his results. Both *Acetobacter xylinum* and *G. suboxydans* in growing, unshaken cultures in 2% glycol-yeast water oxidized the substrate with the formation of acetoin, which was identified as the phenylosazone (mp, 243 to 245 C). A confirmation of our view that the meso form was used is found in the author's statement that the acetoin formed was dextrorotatory. Had he used the D,L mixture, he would probably have obtained the (−) acetoin. The yields were 77% for *G. suboxydans* and 49% for *A. xylinum*, undoubtedly due to poor aeration conditions.

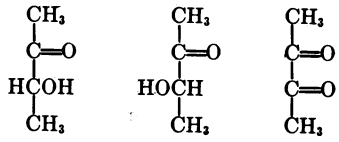
Several other authors have confirmed these results. Grivsky (16) used *A. xylinum* and *M. aceti*. The latter strain was the most active. The oxidation did not go to completion, owing to poor aeration conditions. L(+) acetoin was identified by boiling point, refractive index, several derivatives, etc. Fulmer, Underkofler, and Bantz (14; *see also* 13) worked out a good preparative method with *G. suboxydans*. The glycol was a very poor carbon source for growth, and the culture could not be carried beyond the fifth

transfer on a glycol yeast extract medium. The addition of a low concentration of an assimilable substrate permitted continuous subculture and rapid oxidation of the glycol. Also, Federico (11) succeeded in a quantitative conversion with *A. aceti*, although only small amounts were used (11 mg per 100 ml).

As a systematic investigation on the capacity of meso-2,3-butanediol oxidation by acetic acid bacteria was lacking, such a study was carried out in our laboratory (21, 41). Resting cells of *Gluconobacter* and of *A. liquefaciens* oxidized this substrate to acetoin. All the other strains of the mesoxydans and suboxydans groups of *Acetobacter* oxidized this substrate far beyond the acetoin stage, suggesting that enzymes for the breakdown of L(+) acetoin are present in these bacteria and are lacking in *Gluconobacter* and *A. liquefaciens*. Strains of the peroxydans group of *Acetobacter* oxidized but very slowly. Visser 't Hooft had already reported that *A. rancens* oxidized 2,3-butanediol, and afterwards consumed the acetoin formed.

Acetoin

The results on the oxidation of the compounds shown below are not yet clear-cut.



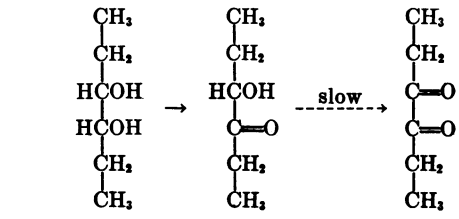
D(−)Acetoin L(+)Acetoin Diacetyl

According to Kling (26), acetoin (possibly his D,L-mixture) resisted attack by *M. aceti*. According to Grivsky (16), *M. aceti* did not attack L(+) acetoin. D(−) Acetoin would be oxidized to diacetyl.

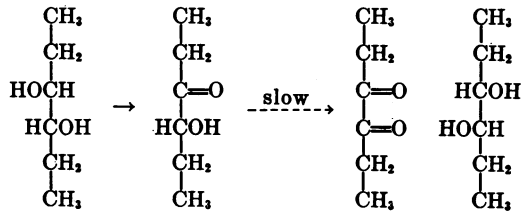
If the above-mentioned results of Verloove (41) and of Kersters and De Ley (21) are to be considered as oxidation of meso-2,3-butanediol by way of L(+) acetoin, then the latter compound would be oxidized by nearly all strains of the mesoxydans and the oxydans groups of *Acetobacter*. Furthermore, it has been shown by De Ley (8) that many strains of both groups are able to produce acetoin from pyruvate by means of the acetaldehyde and the α-acetolactate pathways. The acetoin formed is mainly D(−); it accumulates in appreciable amounts and it is not oxidized further by these strains.

3,4-Hexanediols

Van Risseghem (39) had prepared both the meso form and the racemic mixture of this 3,4-diol and proposed the configurations shown below based on the differences in mp (89.7 C for the meso form and 20.9 C for the racemic mixture).



Meso-3,4-hexanediol L(+) Ethylpropionylcarbinol Dipropionyl



D(+)-3,4-hexanediol D(−) Ethylpropionylcarbinol Dipropionyl L(−)-3,4-hexanediol

She sought to confirm this hypothesis by the application of the Rule of Bertrand, expecting only the meso form to be oxidized. Bertrand's strain of *A. xylinum*, as well as *M. aceti*, were used after growth for up to 2 months at 27 to 31 C in unshaken cultures in yeast extract−2% glycol medium. *M. aceti* was much more active than the sorbose bacterium. Meso-3,4-hexanediol was readily oxidized to L(+)-ethylpropionylcarbinol. However, the oxidation did not go to completion, about 60% of the glycol remaining unchanged, undoubtedly owing to poor aeration conditions; 49.5 g of the diol yielded 11 g of the ketol (about 22%). Interestingly enough, also 1.5 g (3%) of dipropionyl was isolated, showing that a slow oxidation of the L(+) carbinol occurred. That better aeration would have improved the yield was shown in our experiments (21), in which resting cells of *G. suboxydans* oxidized the substrate in a few hours with an oxygen uptake corresponding to the quantitative conversion into the L(+) carbinol. However, contrary to the Rule of Bertrand, one of the optically active isomers was also oxidized. Van

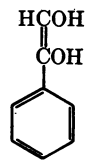
Risseghem (39) found that the D(+) form was attacked. The conversion into D(−) ethylpropionylcarbinol was nearly complete with *M. aceti*, and a small amount of dipropionyl was also formed. *A. xylinum* oxidized only part of the substrate. L(−)3,4-Hexanediol was not attacked.

2,5-Hexanediol

Resting cells of *G. suboxydans* (ATCC 621) oxidized this substrate ($CH_3 \cdot CHOH \cdot CH_2 \cdot CH_2 \cdot HCOH \cdot CH_3$) with the uptake of 0.9 mole of O_2 per mole of substrate (7). A similar result was obtained with our strain of *G. suboxydans*: 0.8 mole of O_2 per mole of substrate (21). The end product has not yet been identified but is likely to be 2,5-diketohexane ($CH_3 \cdot CO \cdot CH_2 \cdot CH_2 \cdot CO \cdot CH_3$).

Styrene Glycol

Styrene glycol



was not oxidized by resting cells of *G. suboxydans* (7).

OTHER GLYCOLS

1,2-Butanediol ($CH_2OH \cdot HCOH \cdot CH_2 \cdot CH_3$), 1,2 - pentanediol ($CH_2OH \cdot HCOH \cdot CH_2 \cdot CH_2 \cdot CH_3$), and 1,2-hexandiol ($CH_2OH \cdot HCOH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_3$) were not oxidized by unshaken growing cultures of *A. xylinum* (26, 6), *M. aceti* (26), and *G. suboxydans* (6).

The results with these three substrates are open to doubt, because they were all obtained with unshaken cultures, under conditions of poor aeration, and with the glycol as main carbon source. These experiments deserve to be repeated either with resting cells and good aeration or with well-aerated growing cultures containing in addition a different, effective carbon source. It would not be surprising to find oxidation under these improved conditions. The eventual end product cannot easily be predicted, since these substrates contain both a primary and a secondary alcohol function. In analogy with 1,2-propanediol, one would expect the secondary OH group to be oxidized.

1,2,4 - Butanetriol ($CH_2OH \cdot HCOH \cdot CH_2 \cdot CH_2OH$) was slowly oxidized by resting cells of *G. suboxydans* (7) with an oxygen uptake of over 0.6 mole of O_2 per mole of substrate. This compound did not support growth. The end product of the oxidation is unknown and hard to predict in view of its similarity to both 1,2-propanediol and 1,3-butanediol.

1,3-Pentanediol ($CH_2OH \cdot CH_2 \cdot HCOH \cdot CH_2 \cdot CH_3$) was oxidized by resting cells of *G. suboxydans*, with an O_2 uptake of 0.65 mole per mole of substrate (7). The end product is unknown.

ENZYMOLOGY OF GLYCOL OXIDATION

This aspect was studied extensively with *G. suboxydans* (21; Kersters and De Ley, *unpublished data*). At least four enzymes appear to be involved in the oxidation of aliphatic glycols by acetic acid bacteria: a soluble nicotinamide adenine dinucleotide (NAD)-linked primary alcohol dehydrogenase, a soluble NAD-linked secondary alcohol dehydrogenase, and at least two particulate oxidative systems. The soluble enzymes were prepared by ultrasonic disruption of the cells and elimination of the cell debris and the ultramicroscopic particulate fraction by centrifugation at 100,000 × *g*. The enzymes were further purified by fractionation and by column chromatography on diethylaminoethyl (DEAE)-cellulose (21). Neither of the purified soluble dehydrogenases oxidized polyols, such as mannitol, sorbitol, glycerol etc., showing that this aspect of glycol oxidation has no connection with the Rule of Bertrand.

Soluble NAD-Linked Primary Alcohol Dehydrogenase

The enzyme did not react with NAD phosphate (NADP). Its specificity is represented in Table 2. The enzyme seems to require the $>CH \cdot CH_2OH$ group. The fact that methanol is not oxidized shows that two carbons are required. The addition of a polar group on or in the vicinity of the C-2 decreases or can completely inhibit enzyme activity. The presence of an OH group or a second CH_2OH group at C-2 decreases or completely prevents enzyme action. The harmful effect of the second OH group is illustrated in the series of ω-diols from 1,3-propanediol to 1,7-heptanediol, in which the enzyme activity increases with the chain length: the more the second CH_2OH group is removed from the one

TABLE 2. *Substrate specificity of two purified soluble enzymes, isolated from Gluconobacter oxydans (suboxydans)**

Substrate	Soluble NAD-linked primary alcohol dehydrogenase	Soluble NAD-linked secondary alcohol dehydrogenase
Methanol	0	0
Ethanol	67	0
n-Propanol	100*	0
n-Butanol	17	0
iso-Butanol	3	0
n-Hexanol	16	0
Allylalcohol	8	0
Ethylene glycol	28	
Ethylene glycol monoethyl ether	17	
Diethylene glycol	0	
Triethylene glycol	0	
1,3-Propanediol	22	0
2-Ethyl-2-nitro-1,3-propanediol	0	
2-Ethyl-2-amino-1,3-propanediol	0	
D,L-1,3-Butanediol	2	0
1,4-Butanediol	25	0
1,5-Pentanediol	39	0
1,6-Hexanediol	42	0
1,7-Heptanediol	67	0
sec-Propanol		1
sec-Butanol	0	12
Cyclopentanol	0	2
Cyclohexanol		4
Cycloheptanol		30
Cyclooctanol		29
tert-Butanol	0	0
D,L-1,2-Propanediol	0	9
L(+)1,2-Propanediol	0	17
Meso-2,3-butanediol	0	100*
D,L-2,3-Butanediol	0	195
Meso-3,4-hexanediol	0	10
(−)3,4-Hexanediol	0	64
Glycerol	0	0
Meso-erythritol	0	0
Na D,L-lactate	0	0
Na D,L-β-hydroxybutyrate		0
Na phosphoglycerate		0

* Relative oxidation rates were expressed against *n*-propanol or meso-2,3-butanediol arbitrarily put at 100. For methods, see reference 21.

to be attacked, the better the enzyme works. When the C-2 is completely surrounded by polar groups, as in 2-ethyl-2-nitro-1,3-propanediol, 2-ethyl - 2 - amino - 1,3 - propanediol, pentaerythritol, or tris(hydroxymethyl)aminomethane (tris), the substrate is not attacked. Etherification of the second primary alcohol group (as in ethylene glycol monomethylether, and di- and triethylene glycol) has a further deleterious effect.

Soluble NAD-Linked Secondary Alcohol Dehydrogenase

This is likewise an enzyme with rather wide specificity (Table 2). The presence of a second adjacent −OH group improves enzyme activity. A third −OH group, a C=O, or a COOH group in the molecule, on the other hand, is deleterious. Lamborg and Kaplan (27) briefly reported on the presence of an enzyme in *G. suboxydans* which oxidized 1,2-propanediol rather strongly and glycerol weakly with NAD and which they called vic-glycol dehydrogenase. Goldschmidt and Krampitz (15) reported, also very briefly, that a NAD-linked enzyme for ethylene glycol and another one for 1,2-propanediol and 2,3-butanediol were present in *G. suboxydans*. In both papers, the substrate specificity of these enzymes was not pursued. It seems obvious that these enzymes are merely our primary and secondary alcohol dehydrogenase.

Particulate Enzymes

The particulate fraction can readily be prepared, after ultrasonic breakage of the cells and elimination of the cell debris by low-speed centrifugation, by ultracentrifugation at 100,000 × *g*. Previous experience with this fraction (10) makes it likely that these enzymes are localized on the cell envelope (probably the cytoplasmic membrane). This fraction oxidized not only all primary and secondary alcohol functions investigated but many other compounds as well (hexoses, pentoses, polyols, aldehydes, etc.). Arcus and Edson (1) had already reported that a particulate fraction from *Acetobacter suboxydans* oxidized polyols with the Bertrand-Hudson configuration. Glycols or other substrates were not investigated. Studies with the particulate fraction itself are not suitable for demonstrating and separating different enzymes, as these are tightly linked to an insoluble matrix. Some success in separation was obtained by releasing the enzymes with the detergent Triton X100 (Rohm & Haas Co., Philadelphia, Pa.; Kersters and De Ley, *unpublished data*). Therefore, a suspen-

sion of washed particles was treated with 0.5% Triton X100 in 0.025 M phosphate buffer with 10^{-4} M ethylenediaminetetraacetic acid (EDTA) at pH 7.6. The supernatant, after high-speed centrifugation at 100,000 × *g*, was freed from nucleic acids with 0.05 M $MnCl_2$, and the active 40 to 45% saturated ammonium sulfate fraction was collected. The solubilized enzymes were further purified by centrifugation in a 5 to 30% sucrose density gradient at 25,000 rev/min for 8 hr. The peak of the alcoholdehydrogenases activity was used for further enzyme studies. This fraction oxidizes numerous compounds as shown in Table 3. From analysis of the behavior of several substrates in different conditions, it appears that the particulate fraction would con-

TABLE 3. *Oxidation of several substrates by a purified enzyme released from the particulate fraction by Triton X100**

Substrate	Reaction rate	Substrate	Reaction rate
Primary alcohols		Compounds with secondary alcohol function	
Methanol	10	sec-Butanol	5
Ethanol	100	sec-Propanol	32
n-Propanol	110	meso-2,3-Butanediol	6
n-Butanol	110	meso-3,4-Hexanediol	9
n-Amylalcohol	100	2,5-Hexanediol	11
n-Hexanol	110	Cyclopentanol	21
n-Octylalcohol	90	Cyclohexanol	13
iso-Butanol	60	Cycloheptanol	11
Allylalcohol	100	Cyclooctanol	14
Glycols with primary alcohol function		D,L-Lactate	4
1,2-Ethyleneglycol	30	Polyols	
1,3-Propanediol	70	Glycerol	1
1,4-Butanediol	80	meso-Erythritol	0
1,5-Pentanediol	80	L-Threitol	0
1,6-Hexanediol	90	D-Arabitol	2
1,7-Heptanediol	140	L-Arabitol	4
1,4-Butinediol	4	meso-Ribitol	3
D,L-1,3-Butanediol	60	meso-Xylitol	2
Ethyleneglycol monoethylether	24	D-Mannitol	0
Diethyleneglycol	1	L-Mannitol	0
Triethyleneglycol	18	D-Glucitol	0
D,L-1,2-Propanediol	9	L-Glucitol	0
Pentaerythritol	0	L-Iditol	0
1,2,6-Hexanetriol	16	D-Iditol	0
2-Amino-2-ethyl-1,3-propanediol	8	meso-Galactitol	0
2-Nitro-2-ethyl-1,3-propanediol	16	meso-Allitol	0
Aromatic alcohols		meso-Glycero-guloheptitol	6
Benzylalcohol	10	D-Glycero-D-galactoheptitol	0
Anisylalcohol	32	D-Gluconate	2
Cinnamylalcohol	34	L-Fucitol	16
Coniferylalcohol	18	L-Rhamnitol	23
Aldehyde function		meso-Inositol	0
Acetaldehyde	38	NADH	12
Propionaldehyde	36		
n-Butyraldehyde	40		
D-Glucose	1		

* The system contained 40 μmoles of substrate, 0.3 μmole of 2,6-dichlorophenolindophenol as H acceptor, and enzyme preparation, in 3.5 ml of 0.02 M phosphate buffer (pH 5.6). The decoloration rate of the H acceptor at 560 mμ was recorded with a colorimeter (Beckman model C) and a Varian recorder. The reaction rate with ethanol was arbitrarily taken as 100 (Kersters and De Ley, *unpublished data*).

TABLE 4. *Synopsis of the oxidation of several aliphatic glycols by acetic acid bacteria*

Substrate	End product(s)	Conditions	Organism and reference
	Oxidation of the primary alcohol function		
Ethylene glycol	Glycolic acid	Static growth	*Acetobacter aceti* (4)
		Static growth	*A. pasteurianus*, *A. kuetzingianus* (33)
		Static growth	*A. xylinum*, *A. rancens*, *G. melanogenus*, *G. suboxydans* (42)
		Submerged growth	*Gluconobacter suboxydans* (18, 31)
		Resting cells	Several strains of *Acetobacter* and *Gluconobacter* (41, 21)
		Particle fraction	*G. suboxydans* (21)
	Oxalic acid	Static growth	Several strains of *Acetobacter* (1*a*)
		Static growth	*A. rancens* (42)
Ethylene glycol monomethylether	(α-Methoxyacetic acid)	Resting cells and particle fraction	*G. suboxydans* (21)
Ethylene glycol monoethylether	α-Ethoxyacetic acid	Submerged growth	*G. suboxydans* (18, 31)
Diethylene glycol	β-Hydroxyethoxyacetic acid → diglycolic acid	Submerged growth	*G. suboxydans* (18, 31)
		Particle fraction	*G. suboxydans* (21)
Diethylene glycol monomethylether	2-(2-Methoxy)-ethoxyacetic acid	Submerged growth	*G. suboxydans* (18, 31)
Triethylene glycol	Not identified	Resting cells and particle fraction	*G. suboxydans* (21)
Thiodiethylene glycol	Not identified	Resting cells	*G. suboxydans* (7)
1,3-Propanediol	Hydracrylic acid → malonic acid	Submerged growth	*G. suboxydans* (18, 31)
	Hydracrylic acid	Resting cells	*G. suboxydans*, *A. liquefaciens* (21)
		Particle fraction	*G. suboxydans* (21)
D,L-1,3-Butanediol	D,L-β-Hydroxybutyric acid	Resting cells	Several strains of *Gluconobacter* and *Acetobacter* (21, 41)
		Particle fraction	*G. suboxydans* (21)
2-Butene-1,4-diol	Not identified	Resting cells	*G. suboxydans* (7)
1,4-Butanediol	γ-Hydroxybutyric acid → succinic acid	Resting cells	Several strains of *Gluconobacter* (21, 41)
		Particle fraction	*G. suboxydans* (21)
		Submerged growth	*G. suboxydans* (18, 31)
	Complete oxidation	Resting cells	Several strains of *Acetobacter* (21, 41)
1,5-Pentanediol	δ-Hydroxyvaleric acid → glutaric acid	Resting cells and particle fraction	*G. suboxydans* (21)
1,6-Hexanediol	ϵ-Hydroxycaproic acid → adipic acid	Resting cells and particle fraction	*G. suboxydans* (21)
		Submerged growth	*G. suboxydans* (18, 31)
D,L-1,2,6-Hexanetriol	Not identified	Resting cells	*G. suboxydans* (7, 21)
		Particle fraction	*G. suboxydans* (21)
1,7-Heptanediol	Pimelic acid	Resting cells and particle fraction	*G. suboxydans* (21)

TABLE 4—*cont.*

Substrate	End product(s)	Conditions	Organism and reference
		Oxidation of the secondary alcohol function	
D(−) and L(+)1,2-Propanediol	Acetol	Static growth	*A. xylinum* (23, 24) and several other strains (25)
		Static growth	*G. suboxydans*, *A. xylinum*, *A. rancens* (42)
		Static growth	*A. xylinum*, *A. aceti*, *G. suboxydans* (6, 40)
		Shaking culture	*G. suboxydans* (5)
		Resting cells	Several strains of *Acetobacter* and *Gluconobacter* (21, 41)
		Particle fraction	*G. suboxydans* (21)
D(−)2,3-Butanediol	D(−)Acetoin (fast)	Static growth	*A. xylinum*, *A. aceti* (26, 16)
		Aerated growth	*G. suboxydans* (38)
		Resting cells and particle fraction	*G. suboxydans* (21)
L(+)2,3-Butanediol	D(−)Acetoin (slow)	Static growth	*A. xylinum* (16)
		Resting cells and particle fraction	*G. suboxydans* (21)
meso-2,3-Butanediol	L(+)Acetoin	Static growth	*A. xylinum*, *G. suboxydans* (42)
		Static growth	*A. xylinum*, *A. aceti* (16)
		Aerated growth	*G. suboxydans* (13, 14)
		Resting cells	*Gluconobacter*, *A. liquefaciens* (21, 41)
		Particle fraction	*G. suboxydans* (21)
D(−)Acetoin	Diacetyl	Static growth	*A. aceti* (16)
		Particle fraction	*G. suboxydans* (21)
meso-3,4-Hexanediol	L(+)Ethylpropionylcarbinol	Static growth	*A. xylinum*, *A. aceti* (39)
		Resting cells and particle fraction	*G. suboxydans* (21)
D(+)3,4-Hexanediol	D(−)Ethylpropionylcarbinol	Static growth	*A. aceti*, *A. xylinum* (39)
L(+)Ethylpropionylcarbinol	Dipropionyl (slow)	Static growth	*A. xylinum*, *A. aceti* (39)
D(−)Ethylpropionylcarbinol	Dipropionyl	Static growth	*A. aceti* (39)
2,5-Hexanediol	Not identified	Resting cells	*G. suboxydans* (7)
		Resting cells and particle fraction	*G. suboxydans* (21)
		Unidentified oxidations	
1,2,4-Butanetriol	Not identified	Resting cells	*G. suboxydans* (7)
1,3-Pentanediol	Not identified	Resting cells	*G. suboxydans* (7)

tain at least three different enzymes for the oxidation of alcohol functions: (i) one or more primary alcohol dehydrogenase, (ii) one or more secondary alcohol dehydrogenase, (iii) one or more polyol dehydrogenases. The first two enzymes would be different, as shown from their behavior during solubilization with Triton X100, from the different yield, and from their different sensitivity against the inhibitors, *p*-chloromercuribenzoate, EDTA, and semicarbazide.

The third enzyme is different from the previous ones, as shown by its behavior against Triton X100 and *p*-chloromercuribenzoate. This again shows that there is no connection between the oxidation of aliphatic glycols and the Rule of Bertrand for the polyols. The Triton X100 treatment has thus released the enzymes from the bulk of the insoluble cell hull. Nevertheless, we are led to believe that the apo-enzymes are still linked together as a larger aggregate, as they could not be separated by either density-gradient centrifugation or column chromatography on several ion-exchange resins. A search for the nature of the coenzyme(s) in this fraction revealed only a cytochrome 553, which is reduced by the primary and secondary alcohol functions of several alcohols, glycols, and polyols. This cytochrome is a tightly linked constituent of the enzyme aggregate. No evidence was found for the participation of NAD, NADP, flavines, the usual ubiquinones, or free heavy metals.

By comparing the results of the oxidations by intact cells with those by the particulate fraction, it is evident that the oxidation products were the same in all cases studied. Thus, the oxidations of glycols effected by intact cells are mainly, if not solely, the result of the enzymatic activity of the cytoplasmic membrane.

Table 4 summarizes the results on the oxidation of aliphatic glycols by different strains of acetic acid bacteria.

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