



Properties and mechanisms

Prepared by:



European Remediation Technologies

Dan Bryant, Kristof Van Vooren and Stijn Haghebaert

**Roodborstjeslaan 31
8300 Knokke Heist**

August, 2005

TABLE OF CONTENTS

1.	REDUCTIVE DECHLORINATION AND CAP18™ ENHANCEMENT	1
1.1	Principles of Reductive Dechlorination	1
1.2	Background Hydrogen Demand	2
1.2.1	<i>Dissolved Oxygen</i>	3
1.2.2	<i>Nitrate</i>	3
1.2.3	<i>Iron</i>	4
1.2.4	<i>Sulfate</i>	4
1.2.5	<i>Methanogenesis</i>	4
1.2.6	<i>Oleate Precipitation</i>	5
1.3	CAP18™ Substrate	5
1.3.1	<i>Composition and Microbial Utilization</i>	6
1.3.2	<i>Inhibition of Methanogenesis</i>	6
1.4	Applicable compounds	7

Annexes :

Annex 1 : Reductive dechlorination

Figure 1-1 : PCE reductive dechlorination

Figure 1-2 : Triacylglycerol structure

Figure 1-3 : Structure of representative C18 unsaturated fatty acid

Figure 1-4 : The Beta oxidation cycle for fatty acids

Figure 1-5 : Fatty acid degradation sequence during Beta oxidation

1. REDUCTIVE DECHLORINATION AND CAP18™ ENHANCEMENT

The basic principle behind engineered (or stimulated) bioremediation is to establish appropriate geochemical conditions and provide a food source (substrate) to stimulate growth of natural microbial populations. In turn, these microbes generate hydrogen, which is utilized by a second group of microbes to metabolize the chlorinated hydrocarbons. A number of comprehensive published sources review groundwater microbiology and the basis of engineered bioremediation of chlorinated solvents (e.g., Wiedemeier et al., 1999; United States Environmental Protection Agency [U.S. E.P.A.], 1999, 2000; Chapelle, 2001). This section provides a summary of microbial reductive dechlorination of chlorinated solvents, background hydrogen demand, and how CAP18™ stimulates reductive dechlorination.

1.1 Principles of Reductive Dechlorination

Reductive dechlorination is a reaction in which a chlorinated solvent acts as an electron acceptor and a chlorine atom on the molecule is replaced with a hydrogen atom, resulting in reduction of the chlorinated solvent (Wiedemeier et al., 1999). When this reaction is biologically mediated, the reaction is referred to as halorespiration and the organisms are referred to as halorespirers. The familiar pathway for dechlorination of perchloroethene (PCE) is presented on Figure 1-1 and is based upon the following generalized oxidation-reduction reaction:



where H₂ is molecular hydrogen, C is carbon, Cl is chloride, and H is hydrogen. In this reaction, H₂ is the electron donor and the chlorinated solvent is the electron acceptor. The net stoichiometry of reductive dechlorination is 1 mole of H₂ for every mole of chloride removed.

PCE undergoes a microbial reduction pathway in which one chlorine atom is sequentially replaced with hydrogen, resulting in the formation of trichloroethene (TCE), dichloroethene (primarily the cis-1,2-dichloroethene [cis-DCE] isomer) and vinyl chloride (VC) as intermediate products (Figure 1-1). The first and second degradation steps, from PCE to TCE and from TCE to

cis-DCE, are relatively fast and occur under almost all anaerobic conditions. Subsequent degradation steps from cis-DCE to VC, and VC to ethene, are relatively slower under anaerobic conditions, and both compounds can also be degraded under aerobic conditions.

Halorespiration is believed to account for the majority of chlorinated solvent degradation where natural attenuation is occurring (Wiedemeier et al., 1999). In order for halorespiration of chlorinated solvents to occur, two related conditions must be met: (1) oxidation-reduction potential must be low (reductive dechlorination is energetically favored only after oxygen, nitrate and iron are depleted from the aquifer), and (2) there must be an adequate supply of fermentable substrates (e.g., fatty acids from CAP18™) for production of dissolved hydrogen (hydrogen is the most important electron donor for halorespiration). Reductive dechlorination is most favorable at hydrogen concentrations greater than 1 nanomolar (nM). Thus the basic premise behind biostimulation is to add sufficient fermentable substrate such that the background hydrogen demand is satisfied and that strongly reducing conditions required by the microbes responsible for dehalorespiration are established, and to maintain those conditions to achieve degradation of the target chlorinated hydrocarbons.

1.2 Background Hydrogen Demand

Wiedemeier et al. (1999) proposed that the following chain of events probably occurs at most sites undergoing halorespiration:

- (1) Aerobic bacteria consume nonchlorinated substrates until oxygen is depleted.
- (2) Nitrate-reducing bacteria consume nonchlorinated substrates until nitrate is depleted.
- (3) Iron-reducing bacteria consume nonchlorinated substrates until ferric iron (Fe^{+3}) is depleted.
- (4) Fermentation reactions consume nonchlorinated substrates and generate hydrogen, which is metabolized by halorespirers to dechlorinate solvents; competing reactions include sulfate-reducing bacteria that consume nonchlorinated substrates and sulfate is depleted, and methanogens that consume hydrogen to generate methane.

Thus many hydrogen demands (chemical and biochemical) exist in aquifers, in addition to the demand from halorespirers to dechlorinate the solvents. The most important of these demands are dissolved oxygen, nitrate, ferric iron, sulfate, and under certain conditions, carbon dioxide. In addition to the aquifer demands, the fatty acids hydrolyzed from CAP18™ can be precipitated as oleates by reaction with calcium and magnesium. Each of these demands must be accounted for when evaluating how much CAP18™ is required, and each of the associated analytes can be measured to evaluate the overall redox condition of the aquifer. These background demands are described below.

1.2.1 Dissolved Oxygen

Dissolved oxygen (DO) is the most favored electron acceptor utilized for microbial degradation of organic carbon. Halorespirers are anaerobic bacteria that generally cannot function at DO concentrations greater than about 0.5 mg/L. Aerobic respiration (which is generally not efficient for chlorinated solvents) will dominate at higher concentrations of DO. The reaction of DO with hydrogen is:



where O₂ is molecular oxygen and H₂O is water. Thus 2 moles of H₂ are consumed for reduction of 1 mole of O₂.

1.2.2 Nitrate

After DO is depleted, microbes will use nitrate (NO₃⁻) as an electron acceptor for anaerobic biodegradation. Nitrate reduction is more energetically favorable than reductive dechlorination. In order for reductive dechlorination to occur, NO₃⁻ concentrations must typically be less than approximately 1 mg/L. The reaction of NO₃⁻ with hydrogen is:



where N₂ is molecular nitrogen. Thus 5 moles of H₂ are consumed for reduction of 2 moles of NO₃⁻.

1.2.3 Iron

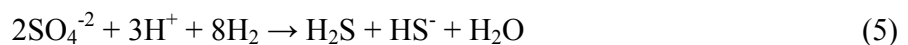
After NO₃⁻ is consumed, the next favored reaction is reduction of Fe⁺³ to ferrous iron (Fe⁺²). Although iron reduction occurs under similar thermodynamic conditions as reductive dechlorination, iron-reducing bacteria are relatively more efficient than haloinspirers. Thus iron reducers rapidly utilize available hydrogen and suppress reductive dechlorination, consuming available hydrogen regardless of the rate of hydrogen production. The stoichiometric reaction for iron reduction is:



thus 1 mole of H₂ is consumed for every 2 moles of Fe⁺³ reduced.

1.2.4 Sulfate

Sulfate (SO₄⁻²) is the next favored electron acceptor. Sulfate reduction is thermodynamically less favorable than reductive dechlorination. However, SO₄⁻² reduction is a very important competitive sink for hydrogen, because of the high concentration relative to chlorinated solvent concentration typically encountered at contaminated sites. The SO₄⁻² reduction reaction with hydrogen is:



where H₂S is hydrogen sulfide and HS⁻ is hydrogen sulfite ion. Thus 4 moles of H₂ are consumed for every 1 mole of SO₄⁻² reduced.

1.2.5 Methanogenesis

Carbon dioxide (CO₂) is the final electron acceptor, used by bacteria to produce methane (CH₄) (a process referred to as methanogenesis). Although haloinspiration is thermodynamically less

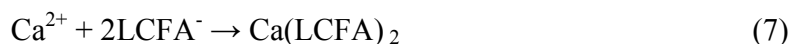
favorable than methanogenesis, reductive dechlorination can occur under methanogenic conditions. The stoichiometric reaction for methanogenesis is:



Thus 4 moles of H₂ are consumed for every 1 mole of CO₂ reduced.

1.2.6 Oleate Precipitation

Calcium and magnesium can react with long-chain fatty acids such as those hydrolyzed from CAP18™ to precipitate oleate, which is a relatively insoluble substrate. This process is the same as soap scum formation from hard water; hard water has high calcium and magnesium concentrations, which react with long-chain fatty acids from the soap to form soap scum. Oleate may slowly dissolve to release the fatty acids and stimulate biodegradation (Yang and McCarty, 2002), although this process has not been demonstrated in aquifers. The stoichiometric reaction for oleate precipitation is:



where Ca²⁺ is calcium ion, LCFA⁻ is a long-chain fatty acid, and Ca(LCFA)₂ is calcium oleate. Thus 1 mole of Ca may precipitate 2 moles of LCFA. Magnesium can behave in the same manner as calcium. This additional precipitation demand must be incorporated into CAP18™ reagent requirements.

1.3 CAP18™ Substrate

CAP18™ is a formulation of food-grade, long-chain fatty acids, refined from natural vegetable oils, and used for environmental remediation solutions. The use of vegetable oils to stimulate anaerobic degradation was first proposed by Hunter and Follett (1994) to address nitrate in ground water. More recently, bench tests (Lee et al., 2000; Zenker et al., 2000) and field pilot tests (Boulicault et al., 2000; Lee et al., 2001; Wiedemeier, 2002) have demonstrated that vegetable oils are very effective for stimulating reductive dechlorination of chlorinated solvents,

and the U.S. Air Force Center for Environmental Excellence has developed a formal protocol for vegetable oil applications (Wiedemeier et al., 2001). This section discusses how vegetable oils, and the fatty acids that comprise vegetable oils, stimulate natural degradation of chlorinated solvents in ground water.

1.3.1 Composition and Microbial Utilization

CAP18™, like the vegetable oils from which it is derived, is a mixture of compounds known as triacylglycerols. Triacylglycerols are compounds comprised of three fatty acid molecules bound by a glycerol backbone (Figure 1-2). Upon injection into an aquifer, the triacylglycerols slowly hydrolyze to release the fatty acids, such as oleic acid (Figure 1-3). The hydrolysis reactions are relatively slow, resulting in sustained production of fatty acids over a very long time period (years). Once released, microbes digest the long-chain fatty acids, derive energy, and produce electrons for reductive dechlorination by a process known as beta-oxidation. Beta-oxidation is a four-step, enzyme-mediated sequence of oxidation, hydrolysis, and thiolysis reactions that occurs in all microbes and animals including humans (Figure 1-4; Novak and Carlson, 1970; Stryer, 1988; Lalman and Bagley, 2000, 2001). During beta-oxidation, the fatty acid molecule is cleaved to produce acetic acid and hydrogen, and the parent fatty acid is shortened by a two-carbon sequence. The shortened fatty acid molecule continues to repeat this cycle, producing a series of shorter-chain fatty acids and ultimately producing either acetic acid or propionic acid. Each cycle produces from two to four hydrogen atoms, which are then available for reduction of organic COCs. For example, beta-oxidation of linoleic acid, a C18 fatty acid and component of CAP18™, produces 9 molecules of acetic acid and 15 molecules of H₂ (Figure 1-5). The hydrogen yield is approximately 1 kg H₂ per 9.7 kg of CAP18™.

1.3.2 Inhibition of Methanogenesis

One of the major limitations with substrate addition to stimulate reductive dechlorination is that the aquifer becomes so anaerobic that microbes known as methanogens compete more effectively for hydrogen than do the dehalorespirers. Under methanogenic conditions, microbes utilize much of the hydrogen produced to generate methane (CH₄) rather than destroy the targeted COCs, thus significantly reducing COC destruction efficiency. Unsaturated C18 fatty acids that primarily

comprise CAP18™ are known to inhibit acetoclastic methanogens, resulting in substantially less methane production (Galbraith et al., 1971; Hanaki et al., 1981; Koster and Cramer, 1987; Rinzema et al., 1994; Lalman and Bagley, 2000, 2001). The result of inhibited methanogenesis is much more efficient use of the hydrogen for destruction of the targeted COCs. Inhibition of methanogenesis does not harm the dehalorespirers or other microbes that reduce targeted COCs, and is a unique attribute of unsaturated C18 fatty acids found in CAP18™.

1.4 Applicable compounds

Chlorinated solvents such as tetrachloroethene (PCE), trichloroethene (TCE), trichloroethane (TCA), and their natural breakdown products such as dichloroethene (DCE); Nitrate; Perchlorate; Explosives; Hexavalent Chromium

Annex 1 :

Reductive dechlorination

Figure 1-1 : PCE reductive dechlorination

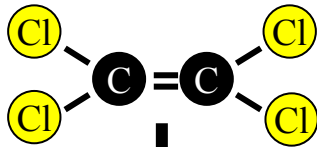
Figure 1-2 : Triacylglycerol structure

Figure 1-3 : Structure of representative C18 unsaturated fatty acid

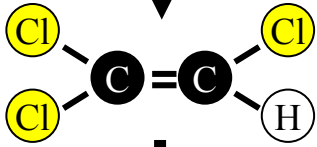
Figure 1-4 : The Beta oxidation cycle for fatty acids

Figure 1-5 : Fatty acid degradation sequence during Beta oxidation

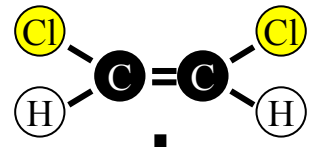
Perchloroethene



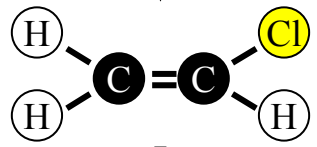
Trichloroethene



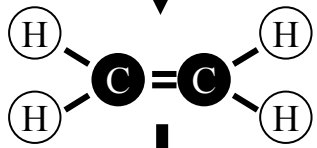
cis-1,2-Dichloroethene



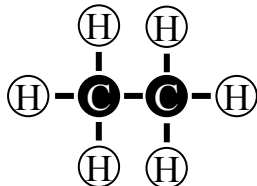
Vinyl Chloride



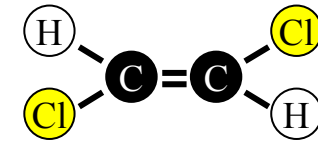
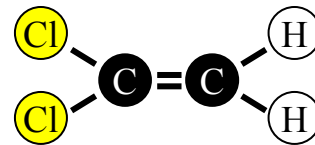
Ethene



Ethane



Limited Biological Reaction



1,1-Dichloroethene

trans-1,2-Dichloroethene

FIGURE 1-1
PCE REDUCTIVE
DECHLORINATION
PATHWAY

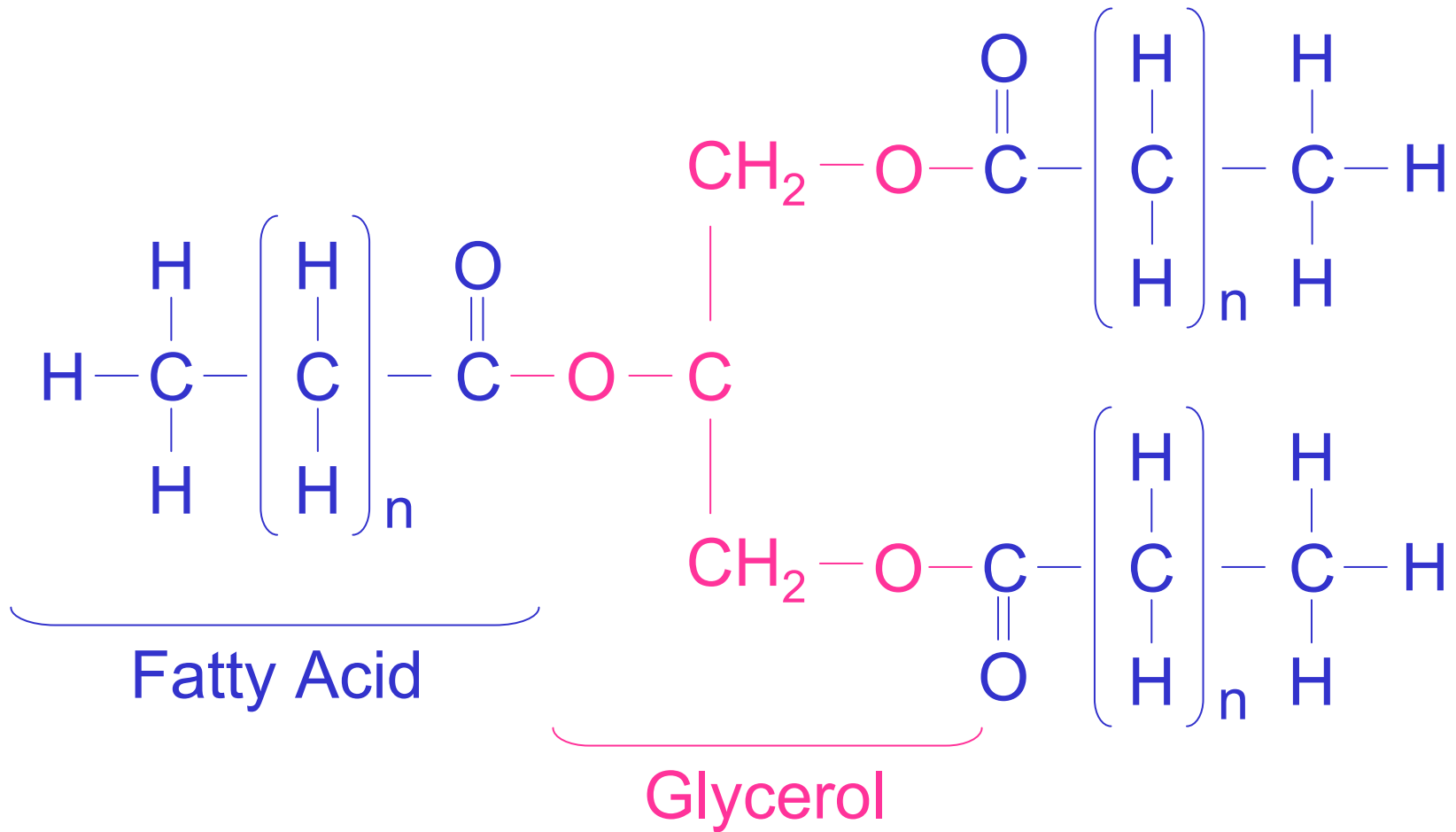
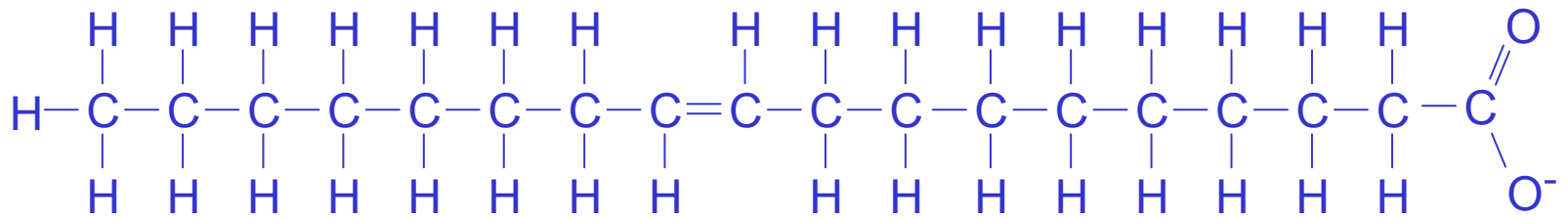


FIGURE 1-2
 TRIACYLGLYCEROL
 STRUCTURE



Oleic Acid (C18:1)

FIGURE 1-3
STRUCTURE OF
REPRESENTATIVE
C18 UNSATURATED
FATTY ACID

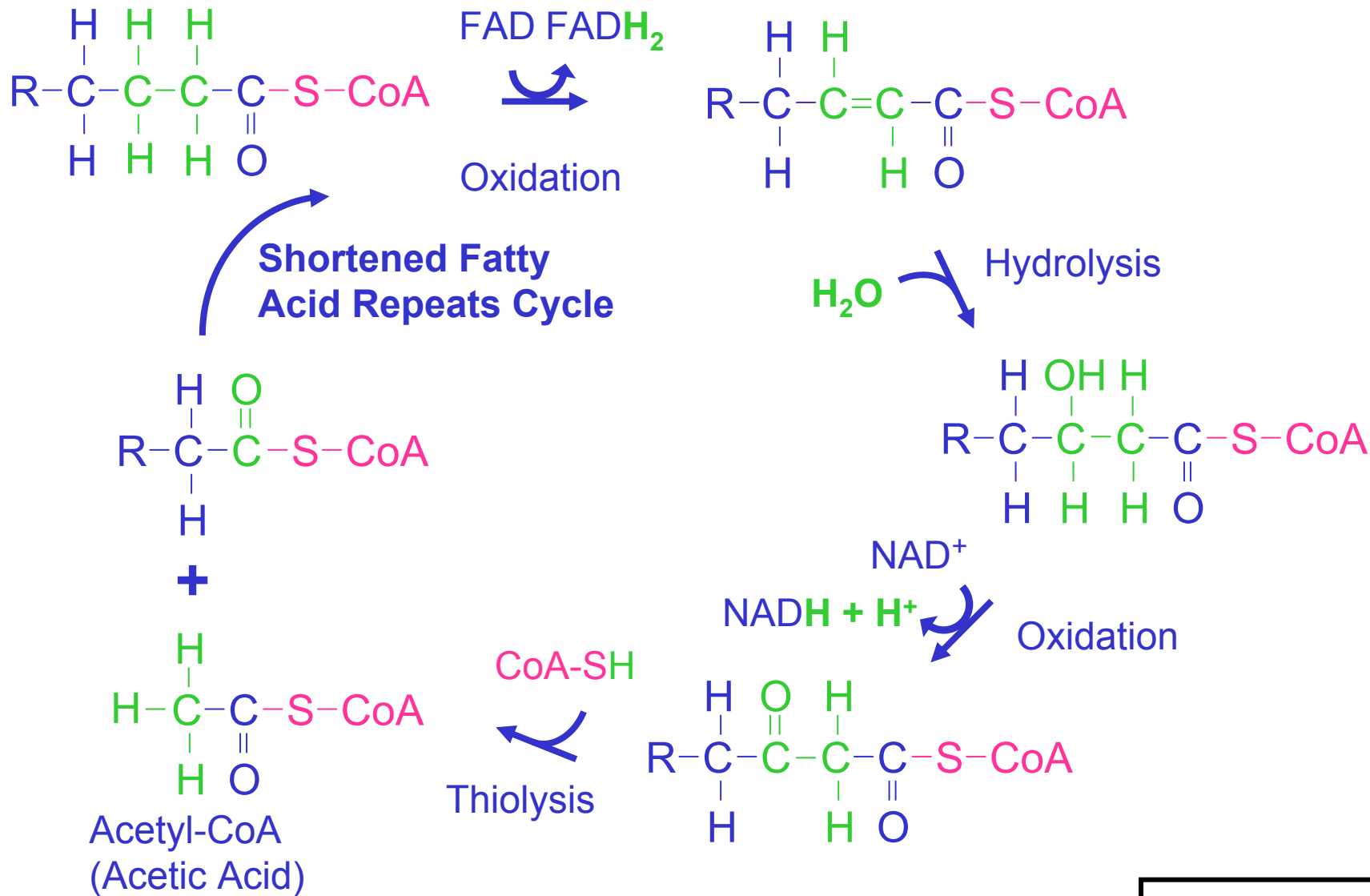
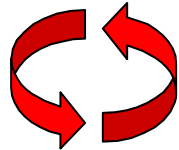
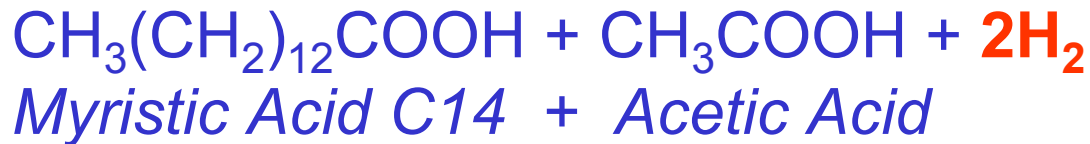
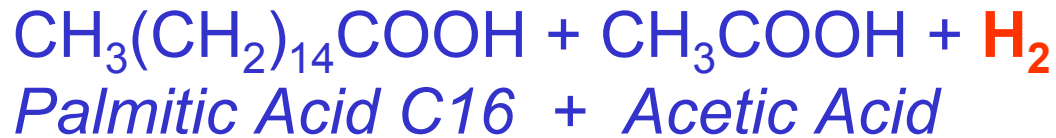
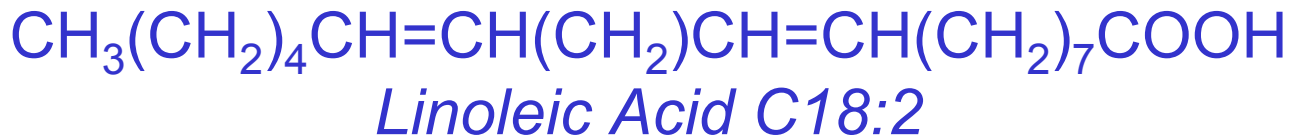


FIGURE 1-4
 THE BETA (β)
 OXIDATION CYCLE
 FOR FATTY ACIDS



6 Additional Cycles



Ultimate Production: 9 molecules of Acetic Acid
15 molecules of H_2

FIGURE 1-5
FATTY ACID
DEGRADATION
SEQUENCE DURING
BETA (β) OXIDATION