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Chapter

Searching for Metabolic Pathways of Anaerobic Digestion: A Useful List of the Key Enzymes

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Abstract

The general scheme of anaerobic digestion is well known. It is a complex process promoted by the interaction of many groups of microorganisms and has four major steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The aim of the study was to prepare a systematized list of the selected enzymes responsible for the key pathways of anaerobic digestion based on the Kyoto Encyclopedia of Genes and Genomes database resource. The list contains (i) key groups of hydrolases involved in the process of degradation of organic matter; (ii) the enzymes catalyzing reactions leading to pyruvate formation; (iii) the enzymes of metabolic pathways of further pyruvate transformations; (iv) the enzymes of glycerol transformations; (v) the enzymes involved in transformation of gaseous or nongaseous products of acidic fermentations resulting from nonsyntrophic nutritional interactions between microbes; (vi) the enzymes of amino acid fermentations; (vii) the enzymes involved in acetogenesis; and (viii) the enzymes of the recognized pathways of methanogenesis. Searching for the presence and activity of the enzymes as well as linking structure and function of microbial communities allows to develop a fundamental understanding of the processes, leading to methane production. In this contribution, the present study is believed to be a piece to the enzymatic road map of anaerobic digestion research.

Keywords: anaerobic digestion, enzymes, hydrolysis, acidogenesis, acetogenesis, methanogenesis, syntrophy, metabolic pathways

1. Introduction

Anaerobic digestion (AD), whose final products are methane and carbon dioxide, is a common process in natural anoxic environments such as water sediments, wetlands, or marshlands. The environments have to be rich in organic matter and poor with other electron acceptors such as nitrate, compounds containing oxidized forms of metals, and sulfate. AD is also common in landfills and wastewater treatment plants and was used by man to produce biogas from waste biomass as an alternative energy source.

AD is a complex process that requires the metabolic interaction of many groups of microorganisms responsible for four closely related major steps. The first one is hydrolysis of complex organic polymers (e.g., polysaccharides, lipids, proteins) to

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monomers (sugars, fatty acids, amino acids). The second step is acidogenesis that results in formation of hydrogen and carbon dioxide as well as nongaseous fermentation products, that is, low-molecular-weight organic acids and alcohols. These products are further oxidized to hydrogen, carbon dioxide, and acetate in acetogenic step that involves mainly syntrophic degradation of nongaseous fermentation products. The fourth step is methanogenesis. Three groups of substrates for methane production and three types of methanogenic pathways are known: splitting of acetate (aceticlastic/acetotrophic methanogenesis); reduction of CO_2 with H_2 or formate and rarely ethanol or secondary alcohols as electron donors (hydrogenotrophic methanogenesis); and reduction of methyl groups of methylated compounds such as methanol, methylated amines, or methylated sulfides (hydrogen-dependent and hydrogen-independent methylotrophic methanogenesis). The two last steps, acetogenesis and methanogenesis, are closely related and involve syntrophic associations between hydrogen-producing acetogenic bacteria and hydrogenotrophic methanogens (**Figure 1**) [1–5].

Recently, there has been a rapid development in culture-independent techniques (meta-omics approaches such as metagenomics, metatranscriptomics, metaproteomics, metabolomics) for exploring microbial communities, which have led to a new insight into their structure and function in both natural environments and anaerobic digesters. The current trends involve the combined use of meta-omic approaches and detailed reactor performance data as well as isotope labeling techniques that allow us to develop a fundamental understanding of the processes occurring in AD. Those activities are aimed to improve

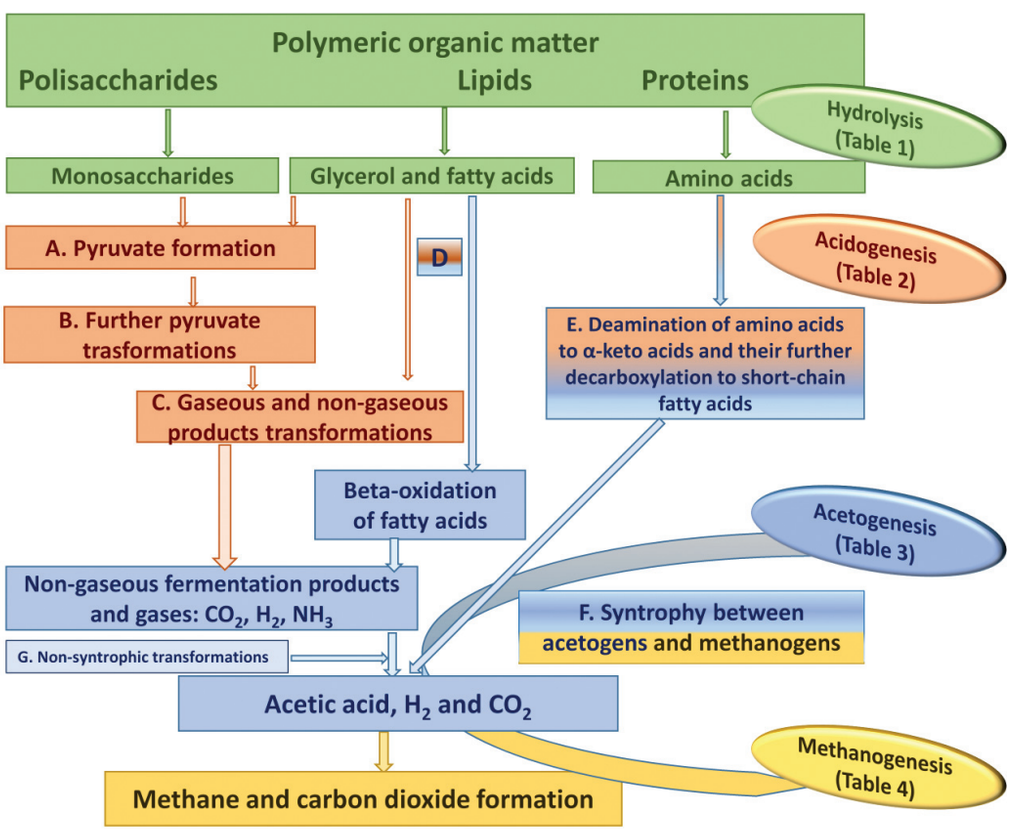

Figure 1.
A scheme of anaerobic digestion of organic matter. Enzymes catalysing specific reactions of AD are presented in ***Tables 1–4****. Thus in Figure 1 there are the links to* ***Tables 1–4****. Furthermore, background colours in the Figure correspond to the background colours of the title rows in the* ***Tables 1–4****: hydrolysis is indicated in green, acidogenesis in orange, acetogenesis in blue and methanogenesis in yellow. A, B, C, D, E refer to the title rows in* ***Table 2****; F, G refer to the title rows in* ***Table 3****.*

biogas production and increase the share of renewable energy in total energy consumption [6–9].

Analysis of many studies on metagenomes of microbial communities from anaerobic digesters shows that (i) contribution of methanogens in the methane-yielding microbial communities is relatively small, below 20%; (ii) the most abundant phyla of bacteria are usually *Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria*; (iii) methanogenic archaea are dominated by acetotrophs or hydrogenotrophs with a certain contribution of methylotrophs; (iv) substrate, operational conditions such as temperature, pH, ammonia concentration, etc. shape the structure, percentage distribution of specific taxons, and functioning of the community of microorganisms; (v) it is important to describe interactions within microbial communities and assign functions in AD steps to specific groups of microbes; and (vi) the majority of sequences are not classified at the genus level confirming that most of the microorganisms are still unrecognized [6, 10–15].

In this contribution, the purpose of the study was to prepare a list of the selected enzymes and their catalyzed reactions, being a specific enzymatic road map of AD metabolic pathways, useful in molecular studies. The available metabolic pathway databases such as KEGG PATHWAY Database [16–18], MetaCyc Metabolic Pathway Database, BioCyc Database Collection [19], and BRENDA—The Comprehensive Enzyme Information System [20] were used to select metabolic pathways dedicated only to AD from hydrolysis to methanogenic steps exerted by microbes.

2. Selected enzymes of anaerobic digestion

Figure 1 shows a scheme of AD and **Tables 1–4** present a summary of the selected enzymes and enzymatic reactions involved in decomposition of organic matter to methane and carbon dioxide. **Tables 1–4** are an extension of **Figure 1**, and in **Figure 1**, there are the links to **Tables 1–4**.

The key groups of hydrolases involved in the process of degradation of organic matter are esterases, glycosidases, and peptidases, which catalyze the cleavage of ester bonds, glycoside bonds, and peptide bonds, respectively (**Table 1**). **Table 1** also includes other classes of hydrolases such as acting on carbon-nitrogen bonds, other than peptide bonds.

In the acidogenic stage of AD, the key step is pyruvate formation from carbohydrates (**Table 2**, Part A) or other compounds and further pyruvate transformations toward short-chain fatty acids and ethanol (**Table 2**, Part B). The Part C of the **Table 2** also considers transformation of gaseous and nongaseous products of acidic fermentations, resulting from nonsyntrophic nutritional interaction between bacteria. The Parts D and E present the enzymes of glycerol and amino acid transformations, respectively. The latter requires syntrophic cooperation between microorganisms.

The enzymes catalyzing oxidation of nongaseous products of acidogenesis mainly butyrate, propionate, acetate, lactate, ethanol including the enzymes of reverse electron transfer (process responsible for energy conservation in syntrophically growing acetogens) are shown in **Table 3**.

The enzymes of the three recognized pathways of methanogenesis such as acetotrophic, hydrogenotrophic, and methylotrophic are listed in **Table 4**.

The data were prepared on the basis of detailed analysis of AD research. The enzyme nomenclature comes from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database resource.

Hydrolytic enzyme	Reaction/process	EC number
Esterases	Acting on ester bonds	EC 3.1
Glycosidases	Acting on glycoside bonds	EC 3.2
	Acting on cellulose	
Cellulase; endo-1,4-beta-D-glucanase	Endohydrolysis of (1 → 4)-beta-D-glucosidic linkages in cellulose, lichenin, and cereal beta-D-glucans	EC 3.2.1.4
Cellulose 1,4-beta-cellobiosidase (nonreducing end)	Hydrolysis of (1 → 4)-beta-D-glucosidic linkages in cellulose and cellotetraose, releasing cellobiose from the nonreducing ends of the chains	EC 3.2.1.91
Beta-glucosidase	Hydrolysis of terminal, nonreducing beta-D-glucosyl residues with release of beta-D-glucose	EC 3.2.1.21
	Acting on hemicellulose	
Endo-1,4-beta-xylanase	Endohydrolysis of (1 → 4)-beta-D-xylosidic linkages in xylans	EC 3.2.1.8
Xylan 1,4-beta-xylosidase	Hydrolysis of (1 → 4)-beta-D-xylans, to remove successive D-xylose residues from the nonreducing termini	EC 3.2.1.37
Mannan endo-1,4-beta-mannosidase	Random hydrolysis of (1 → 4)-beta-D-mannosidic linkages in mannans, galactomannans, and glucomannans	EC 3.2.1.78
Beta-mannosidase	Hydrolysis of terminal, nonreducing beta-D-mannose residues in beta-D-mannosides	EC 3.2.1.25
Alpha-galactosidase	Hydrolysis of terminal, nonreducing alpha-D-galactose residues in alpha-D-galactosides, including galactose oligosaccharides, galactomannans, and galactolipids	EC 3.2.1.22
Alpha-glucuronidase	An alpha-D-glucuronoside + H_2O → an alcohol + D-glucuronate	EC 3.2.1.139
Peptidases	Acting on peptide bonds	EC 3.4
	Other hydrolases	
Hydrolases acting on carbon-nitrogen bonds, other than peptide bonds		EC 3.5
Hydrolases acting on ether bonds		EC 3.3
Hydrolases acting on carbon-carbon bonds		EC 3.7
Hydrolases acting on halide bonds		EC 3.8
Hydrolases acting on phosphorus-nitrogen bonds		EC 3.9
Hydrolases acting on sulfur-nitrogen bonds		EC 3.10
Hydrolases acting on carbon-phosphorus bonds		EC 3.11
Hydrolases acting on sulfur-sulfur bonds		EC 3.12
Hydrolases acting on carbon-sulfur bonds		EC 3.13
Hydrolases acting on acid anhydrides		EC 3.6

Table 1.
The selected enzymes of hydrolytic step of anaerobic digestion [21, 22].

Enzyme	Reaction/process	EC number
A. Pyruvate formation from carbohydrates [23]		
Glycolysis (the Embden-Meyerhof-Parnas pathway)		
Hexose kinase	D-Glucose + ATP ↔ D-glucose-6-phosphate + ADP	EC 2.7.1.1
Phosphoglucose isomerase	D-Glucose 6-phosphate ↔ D-fructose 6-phosphate	EC 5.3.1.9
Phosphofructose kinase	ATP + D-fructose 6-phosphate ↔ ADP + D-fructose 1,6-bisphosphate	EC 2.7.1.11
Fructose-bisphosphate aldolase	Fructose-1,6-bisphosphate ↔ dihydroxyacetone phosphate + glyceraldehyde-3-phosphate	EC 4.1.2.13
Triose phosphate isomerase	Glyceraldehyde 3-phosphate ↔ dihydroxyacetone phosphate	EC 5.3.1.1
Glyceraldehyde-3-phosphate dehydrogenase	D-Glyceraldehyde 3-phosphate + phosphate + NAD^+ ↔ 1,3-bisphosphoglycerate + NADH + H^+	EC 1.2.1.12
Phosphoglycerate kinase	1,3-Bisphosphoglycerate + ADP ↔ 3-phosphoglycerate + ATP	EC 2.7.2.3
Phosphoglycerate mutase	3-Phosphoglycerate ↔ 2-phosphoglycerate	EC 5.4.2.1
Enolase	2-Phospho-D-glycerate ↔ phosphoenolpyruvate + H_2O	EC 4.2.1.11
Pyruvate kinase	Phosphoenolpyruvate + ADP ↔ pyruvate + ATP	EC 2.7.1.40
2-Keto-3-deoxy-6-phosphogluconate (the Entner-Doudoroff pathway)		
Glucose-6-phosphate dehydrogenase	D-glucose 6-phosphate + $NADP^+$ ↔ 6-phospho-D-glucono-1,5-lactone + NADPH + H^+	EC 1.1.1.49
Phosphogluconate dehydrogenase	6-Phospho-D-gluconate + $NAD(P)^+$ ↔ 6-phospho-2-dehydro-D-gluconate + NAD(P)H + H^+	EC 1.1.1.43
2-Keto-3-deoxy-6-phosphogluconate aldolase	2-Dehydro-3-deoxy-6-phospho-D-gluconate ↔ pyruvate + D-glyceraldehyde 3-phosphate	EC 4.1.2.14
B. Further transformations of pyruvate—glycolytic fermentations [23–27]		
Lactate dehydrogenase	Pyruvate + NADH ↔ lactate + NAD^+	EC 1.1.1.27
Pyruvate:ferredoxin oxidoreductase, PFOR	Pyruvate + CoA + oxidized Fd ↔ acetyl-CoA + reduced Fd + CO_2 + H^+	EC 1.2.7.1
NADH:ferredoxin oxidoreductase, NFOR	Oxidized Fd + NADH ↔ reduced Fd + NAD^+ + H^+	EC 1.18.1.3
Ferredoxin hydrogenase	2 reduced ferredoxin + 2 H^+ ↔ H_2 + 2 oxidized ferredoxin	EC 1.12.7.2
Phosphotransacetylase	CoA + acetyl phosphate ↔ acetyl-CoA + phosphate	EC 2.3.1.8
Acetate kinase	ATP + acetate ↔ ADP + acetyl phosphate	EC 2.7.2.1
NAD^+-dependent ethanol dehydrogenase	Acetaldehyde + NADH + H^+ ↔ ethanol + NAD^+ An aldehyde + NADH + H^+ ↔ a primary alcohol + NAD^+	EC 1.1.1.1
Acetaldehyde dehydrogenase	Acetaldehyde + CoA + NAD^+ ↔ acetyl-CoA + NADH + H^+	EC 1.2.1.10
Acetyl-CoA acetyltransferase	2-acetyl-CoA ↔ CoA + acetoacetyl-CoA	EC 2.3.1.9
3-Hydroxybutyryl-CoA dehydrogenase	3-Acetoacetyl-CoA + NADPH + H^+ ↔ 3-hydroxybutanoyl-CoA + $NADP^+$	EC 1.1.1.157
Crotonase 3-OH-butyryl-CoA dehydratase	3-Hydroxybutanoyl-CoA ↔ crotonoyl-CoA + H_2O	EC 4.2.1.55

Enzyme	Reaction/process	EC number
2NADH+ oxidized Fd + crotonyl-CoA → 2 NAD+ reduced Fd + butyryl-CoA catalyzed by butyryl CoA dehydrogenase/electron-transfer flavoprotein complex		
Butyryl-CoA dehydrogenase	A short-chain acyl-CoA + electron-transfer flavoprotein ↔ a short-chain trans-2,3-dehydroacyl-CoA + reduced electron-transfer flavoprotein	EC 1.3.8.1
Butyryl-CoA dehydrogenase/Etf complex	Butanoyl-CoA + 2 NAD^+ + 2 reduced Fd ↔ Crotonoyl-CoA + 2 NADH + 2 oxidized Fd	EC 1.3.1.109
Phosphotransbutyrylase	Butanoyl-CoA + phosphate ↔ CoA + butanoyl phosphate	EC 2.3.1.19
Butyrate kinase	Butanoyl phosphate + ADP ↔ butanoate + ATP	EC 2.7.2.7
PFL—pyruvate formate lyase	Pyruvate + CoA ↔ acetyl-CoA + formate	EC 2.3.1.54
FHL—formate hydrogen lyase	Formate → H_2 + CO_2	EC 1.17.99.7
Pyruvate carboxylase	ATP + pyruvate + HCO_3^- ↔ ADP + phosphate + oxaloacetate	EC 6.4.1.1
Malate dehydrogenase	Malate + NAD^+ ↔ oxaloacetate + NADH + H^+	EC 1.1.1.37
Fumarate hydratase	Malate ↔ fumarate + H_2O	EC 4.2.1.2
Fumarate reductase	Fumarate + a quinol ↔ succinate + a quinone	EC 1.3.5.4
	Fumarate + NADH ↔ succinate + NAD^+	EC 1.3.1.6
Succinyl-CoA synthetase	GTP + succinate + CoA = GDP + phosphate + succinyl-CoA	EC 6.2.1.4
Methylmalonyl CoA mutase	Succinyl-CoA ↔ (R)-methylmalonyl-CoA	EC 5.4.99.2
Methylmalonyl CoA epimerase	(R)-methylmalonyl-CoA ↔ (S)-methylmalonyl-CoA	EC 5.1.99.1
Methylmalonyl-CoA decarboxylase	(S)-methylmalonyl-CoA ↔ propanoyl-CoA + CO_2	EC 4.1.1.41
Propionate-CoA transferase	Acetate + propanoyl-CoA ↔ acetyl-CoA + propanoate	EC 2.8.3.1
C. Transformation of gaseous and nongaseous products of acidic fermentations (the selected examples)		
Transformation of lactate and acetate to butyrate, hydrogen, and carbon dioxide **([28] and cited therein)**		
Lactate dehydrogenases	(S)-lactate + NAD^+ ↔ pyruvate + NADH + H^+	EC 1.1.1.27
	(R)-lactate + NAD^+ ↔ pyruvate + NADH + H^+	EC 1.1.1.28
	Lactate + 2 NAD^+ + 2 reduced Fd ↔ pyruvate + 2 NADH + 2 oxidized Fd See **Table 3**	EC 1.3.1.110
Pyruvate is oxidized to acetyl coenzyme A, which is further routed to acetate and butyrate with hydrogen release. See Part B: Further transformations of pyruvate—glycolytic fermentations		
Transformation of ethanol and acetate to butyrate and hydrogen in Clostridium kluyveri **[29]**		
Acetate kinase	See Part B. Further transformations of pyruvate—glycolytic fermentations	EC 2.7.2.1
Acetyl-CoA acetyltransferase		EC 2.3.1.9
3-Hydroxybutyryl-CoA dehydrogenase		EC 1.1.1.157
3-Hydroxyacyl-CoA dehydratase		EC 4.2.1.55
Butyryl-CoA dehydrogenase/Etf complex		EC 1.3.1.109

Enzyme	Reaction/process	EC number
Acetate CoA-transferase	Acyl-CoA + acetate ↔ a fatty acid anion + acetyl-CoA	EC 2.8.3.8
Reductive carbon monoxide dehydrogenase/acetyl-CoA synthase pathway (reductive CODH/ACS) **[30]**		
NADP-dependent formate dehydrogenase	CO_2 + NADPH ↔ formate + $NADP^+$	EC 1.17.1.10
Formyltetrahydrofolate synthetase	ATP + formate + tetrahydrofolate ↔ ADP + phosphate + 10-formyltetrahydrofolate	EC 6.3.4.3
Methenyltetrahydrofolate cyclohydrolase	10-Formyltetrahydrofolate ↔ 5,10-methenyltetrahydrofolate + H_2O	EC 3.5.4.9
NADP-dependent methylenetetrahydrofolate dehydrogenase	5,10-Methenyltetrahydrofolate + NADPH + H^+ ↔ 5,10-Methylenetetrahydrofolate + $NADP^+$	EC 1.5.1.5
Ferredoxin-dependent methylenetetrahydrofolate reductase	5,10-Methylenetetrahydrofolate + 2 reduced Fd + 2 H^+ ↔ 5-methyltetrahydrofolate + 2 oxidized Fd	EC 1.5.7.1
5,10-Methylenetetrahydrofolate reductase	5,10-Methylenetetrahydrofolate + NAD(P)H + H^+ ↔ 5-methyltetrahydrofolate + $NAD(P)^+$	EC 1.5.1.20
5-Methyltetrahydrofolate: corrinoid/iron–sulfur protein Co-methyltransferase	[Co(I) corrinoid Fe-S protein] + 5-methyltetrahydrofolate ↔ [methyl-Co(III) corrinoid Fe-S protein] + tetrahydrofolate	EC 2.1.1.258
Carbon monoxide dehydrogenase	CO_2 + 2 reduced Fd + 2 H^+ ↔ CO + H_2O + 2 oxidized Fd	EC 1.2.7.4
CO-methylating acetyl-CoA synthase	CO + CoA + [methyl-Co(III) corrinoid Fe-S protein] ↔ acetyl-CoA + [Co(I) corrinoid Fe-S protein]	EC 2.3.1.169
D. Glycerol transformations [31, 32]		
Oxidative pathway		
Glycerol dehydrogenase	Glycerol + NAD^+ ↔ glycerone (dihydroxyacetone) + NADH + H^+	EC 1.1.1.6
Dihydroxyacetone kinase	ATP + glycerone ↔ ADP + glycerone phosphate	EC 2.7.1.29
For further reactions, see Part A: Pyruvate formation		
Reductive pathway		
Glycerol dehydratase	Glycerol ↔ 3-hydroxypropionaldehyde + H_2O	EC 4.2.1.30
1,3-Propanediol dehydrogenase	3-Hydroxypropionaldehyde + NADH + H^+ ↔ 1,3-propanediol + NAD^+	EC 1.1.1.202
E. Amino acids fermentations [33–37]		
Syntrophy with H_2-scavenging microorganism: amino acid degradation involves NAD(P)- or FAD-dependent deamination of amino acids to the corresponding α-keto acids by amino acid dehydrogenases **(EC 1.4.1.X)**: $RCH(NH_4^+)COO^- + H_2O \rightarrow RCOCOO^- + NH_4^+ + H_2$ and further conversion of α-keto acids via oxidative decarboxylation to fatty acids: $RCOCOO^- + H_2O \rightarrow RCOO^- + CO_2 + H_2$ [33]		
Without syntrophy with H_2-scavenging microorganism: **Stickland Reaction—**coupled oxidation-reduction reactions between suitable amino acids (coupled deamination of amino acids); one member of the pair is oxidized (dehydrogenated) and the other is reduced (hydrogenated) [34], for example, Alanine and glycine: alanine + 2 glycine + $3H_2O \rightarrow$ 3 $acetate^-$ + $3NH_4^+$ + HCO_3^- + H^+ Valine and glycine: valine + 2 glycine + $3H_2O \rightarrow$ $isobutyrate^-$ + 2 $acetate^-$ + $3NH_4^+$ + HCO_3^- + H^+ Leucine and glycine: leucine + 2 glycine + $3H_2O \rightarrow$ $isovalerate^-$ + 2 $acetate^-$ + $3NH_4^+$ + HCO_3^- + H^+		

Enzyme	Reaction/process	EC number
Examples of amino acid dehydrogenases catalyzing deamination of amino acids to the corresponding α-keto acids [33]		
Aspartate dehydrogenase	L-aspartate + H_2O + NAD(P)$^+$ ↔ oxaloacetate + NH_3 + NAD(P)H + H^+	EC 1.4.1.21
Valine dehydrogenase	L-valine + H_2O + $NADP^+$ ↔ 3-methyl-2-oxobutanoate + NH_3 + NADPH + H^+	EC 1.4.1.8
Alanine dehydrogenase	L-alanine + H_2O + NAD^+ ↔ pyruvate + NH_3 + NADH + H^+	EC 1.4.1.1
Leucine dehydrogenase	L-leucine + H_2O + NAD+ ↔ 4-methyl-2-oxopentanoate + NH_3 + NADH + H^+	EC 1.4.1.9
Key enzymes of Stickland reaction [34–36]		
Glycine reductase GR pathway (*grd* operon)		
Glycine reductase	Glycine + phosphate + reduced thioredoxin + H^+ ↔ acetyl phosphate + NH_3 + oxidized thioredoxin + H_2O	EC 1.21.4.2
Acetate kinase	Acetyl phosphate + ADP ↔ acetate + ATP	EC 2.7.2.1
Proline reductase PR pathway (*prd* operon)		
D-proline reductase (dithiol)	D-proline + dihydrolipoate ↔5-aminopentanoate (5-aminovalerate) + lipoate	EC 1.21.4.1
Others examples [33]		
Serine dehydratase	L-serine ↔ pyruvate + NH_3 (overall reaction) (1a) L-serine ↔ 2-aminoprop-2-enoate + H_2O (1b) 2-Aminoprop-2-enoate ↔ 2-iminopropanoate (spontaneous) (1c) 2-Iminopropanoate + H_2O ↔ pyruvate + NH_3 (spontaneous)	EC 4.3.1.17
Threonine dehydratase	L-threonine ↔ 2-oxobutanoate + NH_3 (overall reaction) (1a) L-threonine ↔ 2-aminobut-2-enoate + H_2O; (1b) 2-Aminobut-2-enoate ↔ 2-iminobutanoate (spontaneous) (1c) 2-Iminobutanoate + H_2O ↔ 2-oxobutanoate + NH_3 (spontaneous)	EC 4.3.1.19
Detailed pathways of glutamate fermentation via 3-methylaspartate [37]		
Glutamate mutase (methylaspartate mutase)	L-glutamate ↔ L-threo-3-methylaspartate	**EC 5.4.99.1**
Methyl aspartase	L-threo-3-methylaspartate ↔ mesaconate (2-methylfumarate) + NH_3	EC 4.3.1.2
Mesaconase (2-methylmalate dehydratase)	2-Methylfumarate + H2O ↔ (S)-2-methylmalate	4.2.1.34
Citramalate lyase	(2S)-2-hydroxy-2-methylbutanedioate ↔ acetate + pyruvate (S)-2-methylmalate = 2-hydroxy-2-methylbutanedioate	4.1.3.22
For further transformations of pyruvate to acetate and butyrate, see Part B.		
For further transformations of pyruvate to propionate, see Part B.		
Detailed pathway of glutamate fermentation via 2-hydroxyglutarate [37]		
Glutamate dehydrogenase	L-glutamate + H_2O + NAD^+ ↔ 2-oxoglutarate + NH_3 + NADH + H^+	1.4.1.2

Enzyme	Reaction/process	EC number
2-Hydroxyglutarate dehydrogenase	(S)-2-hydroxyglutarate + acceptor ↔ 2-oxoglutarate + reduced acceptor	1.1.99.2
Glutaconate (2-hydroxyglutarate) CoA-transferase	Acetyl-CoA + (E)-glutaconate ↔ acetate + glutaconyl-1-CoA	2.8.3.12
2-Hydroxyglutaryl-CoA dehydratase	**(R)-2-hydroxyglutaryl-CoA ↔ (E)-glutaconyl-CoA + H_2O**	**EC 4.2.1.167**
Glutaconyl-CoA decarboxylase	4-Carboxybut-2-enoyl-CoA ↔ but-2-enoyl-CoA + CO_2	4.1.1.70

Table 2.
The selected enzymes of acidogenic step of anaerobic digestion. A, B, C, D, and E refer to the processes indicated in ***Figure 1****.*

Enzyme	Reaction/process	EC number
F. Acetogenesis dependent on syntrophic relations between microorganisms		
Acetate oxidation by, for example, *Clostridium ultunense*—oxidative carbon monoxide dehydrogenase/acetyl-CoA synthase pathway (oxidative CODH/ACS): $Acetate^- + 4H_2O \rightarrow 2\ HCO_3^- + 4H_2 + H^+$, $\Delta G^{0'} = +104.6$ kJ/mol, with the H_2 consuming methanogen, $\Delta G^{0'} = -31.0$ kJ/mol [38]		
NADP-dependent formate dehydrogenase		See **Table 2**, Part C
Formyltetrahydrofolate synthetase		
Methenyltetrahydrofolate cyclohydrolase		
NADP-dependent methylenetetrahydrofolate dehydrogenase		
Ferredoxin-dependent methylenetetrahydrofolate reductase		
5,10-Methylenetetrahydrofolate reductase		
5-Methyltetrahydrofolate:corrinoid/iron-sulfur protein Co-methyltransferase		
Carbon monoxide dehydrogenase		
CO-methylating acetyl-CoA synthase		
Reverse electron transfer during acetate oxidation has yet to be confirmed. Direct interspecies electron transfer (DIET) is not excluded (Westerholm et al., 2016)		
Acetate oxidation by *Geobacter sulfurreducens:* Acetate oxidation coupled to reduction of fumarate to succinate ($\Delta G^{o'} = -249$ kJ per mol acetate), acetate metabolism proceeds via reactions of the citric acid cycle [39]		
Acetate kinase		See **Table 2**, Part B
Phosphotransacetylase		
Citric acid cycle		
Citrate synthase	Acetyl-CoA + H_2O + oxaloacetate ↔ citrate + CoA	EC 2.3.3.1
Aconitase	Citrate ↔ isocitrate (overall reaction)	EC 4.2.1.3
Isocitrate dehydrogenase ($NADP^+$-dependent)	Isocitrate + $NADP^+$ ↔ 2-oxoglutarate + CO_2 + NADPH + H^+	EC1.1.1.42
2-Oxoglutarate:ferredoxin oxidoreductase	2-Oxoglutarate + CoA + 2 oxidized Fd = succinyl-CoA + CO_2 + 2 reduced Fd + 2 H^+	EC 1.2.7.3
Succinyl-CoA:acetate CoA-transferase	Succinyl-CoA + acetate ↔ acetyl-CoA + succinate	EC 2.8.3.18

Enzyme	Reaction/process	EC number
Succinate dehydrogenase	succinate + a quinone ↔ fumarate + a quinol	EC 1.3.5.1
Fumarate hydratase	(S)-malate ↔ fumarate + H_2O	EC 4.2.1.2
Malate dehydrogenase	(S)-malate + NAD^+ ↔ oxaloacetate + NADH + H^+	EC 1.1.1.37
Butyrate oxidation by *Syntrophomonas wolfei*: $Butyrate^- + 2H_2O \rightarrow 2\ acetate^- + 2H^+ + 2H_2$, $\Delta G^{0'} = +48.3$ kJ/mol, with the H_2 consuming methanogen, $\Delta G^{0'} = -17.3$ kJ/mol [4]		
CoA transferase	Butyrate + acetyl-CoA ↔ butyryl-CoA + acetate	EC 2.8.3.9
Butyryl-CoA dehydrogenase		See **Table 2**, Part B
Crotonase-3-OH-butyryl-CoA dehydratase		
3-Acetyl-CoA acetyltransferase		
Hydroxybutyryl-CoA dehydrogenase		
Phosphotransacetylase		
Acetate kinase		
Butyrate oxidation coupled with a reverse electron transfer that involves electron transfer flavoprotein EtfAB, membrane-anchored electron carrier DUF224 protein, the menaquinone pool in the membrane, a membrane-bound cytochrome, NADH:hydrogenase/formate-dehydrogenase complex (NDH/HYD1/FDH-1 complex), Rnf (proton-translocating ferredoxin:NAD^+ oxidoreductase) [40]		
Propionate oxidation by *Syntrophobacter wolinii*: $Propionate^- + 3H_2O \rightarrow acetate^- + HCO_3^- + H^+ + 3H_2$, $\Delta G^{0'} = +76.0$ kJ/mol, with the H_2 consuming methanogen, $\Delta G^{0'} = -22.4$ kJ/mol [4]		
Pyruvate carboxylase		See **Table 2**, Part B
Malate dehydrogenase		
Fumarate hydratase		
Fumarate reductase		
Succinate dehydrogenase	Succinate + a quinone ↔ fumarate + a quinol	EC 1.3.5.1
Succinyl-CoA synthetase		See **Table 2**, Part B
Methylmalonyl CoA mutase		
Methylmalonyl CoA epimerase		
Methylmalonyl-CoA decarboxylase		
Propionate-CoA transferase		
Propionate oxidation coupled with a reverse electron transfer that involves menaquinone, proteins encoded by cytochrome c homologous genes, cytochrome b:quinone oxidoreductases, formate dehydrogenases, hydrogenases including confurcating [FeFe]-hydrogenases [41]		
Six syntrophy-specific functional domains found in the genomes of the butyrate- or propionate-oxidizing syntrophs [42]	**InterPro number**	
Extra-cytoplasmic formate dehydrogenase (FDH) alpha subunit, EC 1.17.1.9	IPR006443	
FdhE-like protein—tightly connected with FDH	IPR024064	

Enzyme	Reaction/process	EC number
FDH accessory protein—tightly connected with FDH	IPR006452	
CapA—a membrane-bound complex, a protein involved in capsule or biofilm formation that may facilitate syntrophic growth (*also present in acetate-oxidizers*)	IPR019079	
FtsW, RodA, SpoVE—membrane-integrated proteins involved in membrane integration, cell division, sporulation, and shape determination	IPR018365	
Ribonuclease P involved in tRNA maturation	IPR020539	
Functional domains involved in electron transfer identified by [42]	**InterPro number**	
Cytoplasmic FDH	IPR027467, IPR006655, IPR006478, IPR019575, IPR001949	
Extracytoplasmic FDH	IPR006443	
Formate transporter	IPR000292, IPR024002	
Fe-Fe hydrogenase	IPR004108, IPR009016, IPR003149, IPR013352	
NiFe hydrogenase	IPR001501, IPR018194	
Rnf complex: 2 reduced Fd + NAD^+ + H^+ + Na^+ ↔ 2 oxidized Fd + NADH + Na^+ (EC 1.18.1.8)	IPR007202, IPR010207, IPR026902, IPR010208, IPR004338, IPR011303, IPR007329	
Ech complex: 2 reduced Fd + $NADP^+$ + H^+ ↔ 2 oxidized Fd + NADPH (EC 1.18.1.2)	IPR001750, IPR001516, IPR001694, IPR006137, IPR001268, IPR012179, IPR001135	
Etf alpha, Etf beta, Bcd (Butyryl-CoA dehydrogenase): see **Table 2**, Part B (EC 1.3.1.109)	IPR014731, IPR012255, IPR006089, IPR009075, IPR006092, IPR006091, IPR013786, IPR009100	
Cytochromes: c cIII b561 b5	IPR023155, IPR024673 IPR020942, IPR002322 IPR016174, IPR000516 IPR001199	
DUF224 protein complex	IPR003816, IPR004017, IPR023234	
Lactate oxidation by *Desulfovibrio vulgaris*: Lactate$^-$ + H_2O → acetate$^-$ + CO_2 + 4 H_2, $\Delta G^{0'}$ = −8.8 kJ/mol with the H_2 consuming methanogen, $\Delta G^{0'}$ = −74.2 kJ/mol [43]		
Lactate dehydrogenase		See **Table 2**, Part B
Pyruvate:ferredoxin oxidoreductase		
Phosphate acetyltransferase		
Acetate kinase		
Alcohol dehydrogenase		
Lactate oxidation coupled with a reverse electron transfer that involves the membrane-bound Qmo complex, cytochromes, hydrogenases (Coo, Hyn, Hyd, Hys), formate dehydrogenases, menaquinone, membrane-bound Qrc complex [43, 44]		

Enzyme	Reaction/process	EC number
Ethanol oxidation by *Pelobacter carbinolicus* Ethanol + H_2O → acetate$^-$ + H^+ + $2H_2$, $\Delta G^{0'}$ = + 9.6 kJ/mol with the H_2 consuming methanogen, $\Delta G^{0'}$ = − 56 kJ/mol [4]		
NAD^+-dependent ethanol dehydrogenase		See **Table 2**, Part B
Acetaldehyde dehydrogenase (acetylating)		
Nonacetylating acetaldehyde dehydrogenase	An aldehyde + NAD^+ + H_2O ↔ a carboxylate + NADH + H^+	EC 1.2.1.3
Phosphotransacetylase		See **Table 2**, Part B
Acetate kinase		
Ethanol oxidation coupled with a reverse electron transfer that involves membrane-bound ion-translocating ferredoxin:NAD^+ oxidoreductase, formate dehydrogenases, and confurcating hydrogenases [1, 45]		
G. Acetogenesis independent on syntrophic relations between microorganisms		
Ethanol oxidation by *Acetobacterium woodii*: 2 ethanol + 2 CO_2 → 3 acetate—75.4 kJ/mol [46]		
Bifunctional acetaldehyde-CoA/alcohol dehydrogenase	Ethanol + NAD^+ → acetaldehyde + NADH + H^+ acetaldehyde + NAD^+ + CoA → acetyl-CoA + 2 NADH + H^+ Ethanol is oxidized to acetyl-CoA in a two-step reaction by a bifunctional acetylating ethanol/aldehyde dehydrogenase	[EC:1.2.1.10 1.1.1.1]
Acetyl-CoA is transformed to acetate with the release of ATP		See **Table 2**, Part B
Reduction of ferredoxin by NADH by reverse electron flow in a reaction catalyzed by Rnf complex		See Part F
Carbon dioxide is reduced to acetate via the Wood-Ljungdahl pathway		See **Table 2**, Part C
Lactate oxidation by *Acetobacterium woodii*: 2 lactate → 3 acetate—61 kJ/mol [47]		
Lactate dehydrogenase	Lactate + 2 NAD^+ + 2 reduced Fd ↔ pyruvate + 2 NADH + 2 oxidized Fd The enzyme uses flavin-based electron confurcation to drive endergonic lactate oxidation with NAD^+ as oxidant at the expense of simultaneous exergonic electron flow from reduced ferredoxin to NAD^+	EC 1.3.1.110
Pyruvate is transformed to acetyl-CoA and further to acetate with the release of ATP		See **Table 2**, Part B
Reduction of ferredoxin by NADH by reverse electron flow in a reaction catalyzed by Rnf complex		See Part F
Carbon dioxide is reduced to acetate via the Wood-Ljungdahl pathway		See **Table 2**, Part C

Table 3.
The selected enzymes of acetogenic step of anaerobic digestion. F and G refer to the processes indicated in ***Figure 1****.*

Enzyme	Reaction/process	EC number
MFR—methanofuran, H-S-CoM—coenzyme M, H-S-CoB—coenzyme B, H_4MPT—tetrahydromethanopterin, F_{420}—5'deazaflavin, H_4SPT—tetrahydrosarcinapterin		
Hydrogenotrophic pathway		
Formylmethanofuran dehydrogenase	CO_2 + MFR + 2 reduced Fd + $2H^+$ ↔ formyl-MFR + H_2O + 2 oxidized Fd	EC 1.2.7.12
Formylmethanofuran-H_4MPT formyltransferase	Formyl-MFR + H_4MPT ↔ MFR + formyl-H_4MPT	EC 2.3.1.101
Methenyl-H_4MPT cyclohydrolase	Formyl-H_4MPT + H^+ ↔ methenyl-H_4MPT + H_2O	EC 3.5.4.27
F_{420}-dependent methylene-H_4MPT dehydrogenase	Methenyl-H_4MPT + reduced F_{420} ↔ methylene-H_4MPT + oxidized F_{420}	EC 1.5.98.1
H_2-forming methylene-H_4MPT dehydrogenase	Methenyl-H_4MPT + H_2 ↔ methylene-H_4MPT + H^+	EC 1.12.98.2
F_{420}-dependent methylene-H_4MPT reductase	Methylene-H_4MPT + reduced F_{420} ↔ CH_3-H_4MPT + oxidized F_{420}	EC 1.5.98.2
Methyl-H_4MPT:coenzyme M methyltransferase	Coenzyme M + methyl-H_4MPT + 2 Na+/in ↔ 2-methyl-coenzyme M + 2 Na+/out + H_4MPT	EC 2.1.1.86
Methyl-CoM reductase	CH_3-S-CoM + H-S-CoB ↔ CoM-S-S-CoB + CH_4	EC 2.8.4.1
Heterodisulfide reductase	CoM-S-S-CoB + dihydromethanophenazine ↔ CoB + CoM + methanophenazine	EC 1.8.98.1
Acetotrophic pathway		
Acetate kinase-phosphotransacetylase system in *Methanosarcina*; acetate thiokinase in *Methanosaeta*	Acetate + CoA ↔ acetyl-CoA + H_2O	EC 2.7.2.1 EC 2.3.1.8 EC 6.2.1.1
CO-methylating acetyl-CoA synthase	Acetyl-CoA + a [Co(I) corrinoid Fe-S protein] ↔ CO + CoA + [methyl-Co(III) corrinoid Fe-S protein]	EC 2.3.1.169
5-Methyltetrahydrosarcinapterin: corrinoid/iron-sulfur protein Co-methyltransferase	[Methyl-Co(III) corrinoid Fe-S protein] + tetrahydrosarcinapterin ↔ a [Co(I) corrinoid Fe-S protein] + 5-methyltetrahydrosarcinapterin	EC 2.1.1.245
Anaerobic carbon monoxide dehydrogenase	CO + H_2O + 2 oxidized Fd ↔ CO_2 + 2 reduced Fd + 2 H^+	EC 1.2.7.4
Methyl H_4SPT: coenzyme M methyltransferase	CH_3 H_4SPT + H-S-CoM ↔ CH_3-S-CoM + H_4SPT	EC 2.1.1.-
Methyl-CoM reductase	CH_3-S-CoM + H-S-CoB ↔ CoM-S-S-CoB + CH_4	EC 2.8.4.1
Heterodisulfide reductase	CoM-S-S-CoB + dihydromethanophenazine ↔ CoB + CoM + methanophenazine	EC 1.8.98.1
Methylotrophic pathway		
Methanol:corrinoid protein Co-methyltransferase	Methanol + Co(I) corrinoid protein ↔ Methyl-Co(III) corrinoid protein + H_2O	EC 2.1.1.90
[Methyl-Co(III) corrinoid protein]: coenzyme M methyltransferase	Coenzyme M + Methyl-Co(III) corrinoid protein ↔ 2-(methylthio)ethanesulfonate + Co(I) corrinoid protein	EC 2.1.1.246

Enzyme	Reaction/process	EC number
Methylamine:corrinoid protein Co-methyltransferase	Methylamine + [Co(I) methylamine-specific corrinoid protein] ↔ a [methyl-Co(III) methylamine-specific corrinoid protein] + NH_3	EC 2.1.1.248
Dimethylamine:corrinoid protein Co-methyltransferase	Dimethylamine + [Co(I) dimethylamine-specific corrinoid protein] ↔ a [methyl-Co(III) dimethylamine-specific corrinoid protein] + methylamine	EC 2.1.1.249
Trimethylamine:corrinoid protein Co-methyltransferase	Trimethylamine + a [Co(I) trimethylamine-specific corrinoid protein] ↔ a [methyl-Co(III) trimethylamine-specific corrinoid protein] + dimethylamine	EC 2.1.1.249
[Methyl-Co(III) methylamine-specific corrinoid protein]:coenzyme M methyltransferase	[Methyl-Co(III) methylamine-specific corrinoid protein] + CoM ↔ methyl-CoM + a [Co(I) methylamine-specific corrinoid protein]	EC 2.1.1.247
Methyl-CoM reductase	CH_3-S-CoM + H-S-CoB ↔ CoM-S-S-CoB + CH_4	EC 2.8.4.1
Heterodisulfide reductase	CoM-S-S-CoB + dihydromethanophenazine ↔ CoB + CoM + methanophenazine	EC 1.8.98.1

Table 4.
The selected enzymes of methanogenic step of anaerobic digestion [48, 49].

3. Conclusion

Biomass conversion to methane and carbon dioxide is the effect of complex interactions between microorganisms. These processes occur due to the microbial enzymatic machinery involved in specific metabolic pathways. Meta-omic analyses of microbial communities involved in AD reveal (i) dependence of microbial communities on the type of feedstock and operational conditions and (ii) describe interactions within microbial communities and ecophysiological functions of the specific taxa. Searching for the gene presence, gene expression, and protein expression, as well as linking structure and function of microbial communities, allows to develop a fundamental understanding of AD. This chapter is believed to contribute to the studies on the enzymatic road map of anaerobic digestion. However, it is only the tip of the iceberg of processes occurring in the microbial cells/microbial communities.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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