ANAEROBIC DIGESTION *Lecture-I*, BECS-II

Not all micro-organisms must utilize oxygen, and in fact many cann't. Metabolism in the absence of oxygen is called anaerobic. Anaerobic organisms have played a vital role in wastewater treatment through anaerobic digestion, which has been the primary source degrading organic solids in aqueous systems. In recent times, anaerobic organisms are used for stabilization of solid organic residues, to produce METHANE and ORGANIC MANURE. In addition, anaerobic organisms have been used to remove soluble organic matter. Regardless of the objective, however all anaerobic bio-chemical operations share a unique biological feature – the formation of METHANE. This gas is rapidly discharged from the system, because of its low solubility in water resulting in a rather high degree of waste stabilization. This unique feature governs the design and operation anaerobic biochemical operations to such a degree, that the nature of the waste treatment objective is les important than it is in aerobic operations.

Description of Anaerobic operations :

Unlike aerobic operations, which contain diverse microbial communities and complex food chains, anaerobic operations contain communities which are essentially totally bacterial. In spite of this apparent simplicity, interactions among the bacterial species have a severe effect upon system performance. Early attempts to design and control anaerobic operations were hampered by our ignorance of these interactions, which caused the operations to acquire a reputation for being unreliable and difficult to control.

Microbiological and Biochemical characteristics :

The multiple nature of anaerobic operations is depicted in fig.1

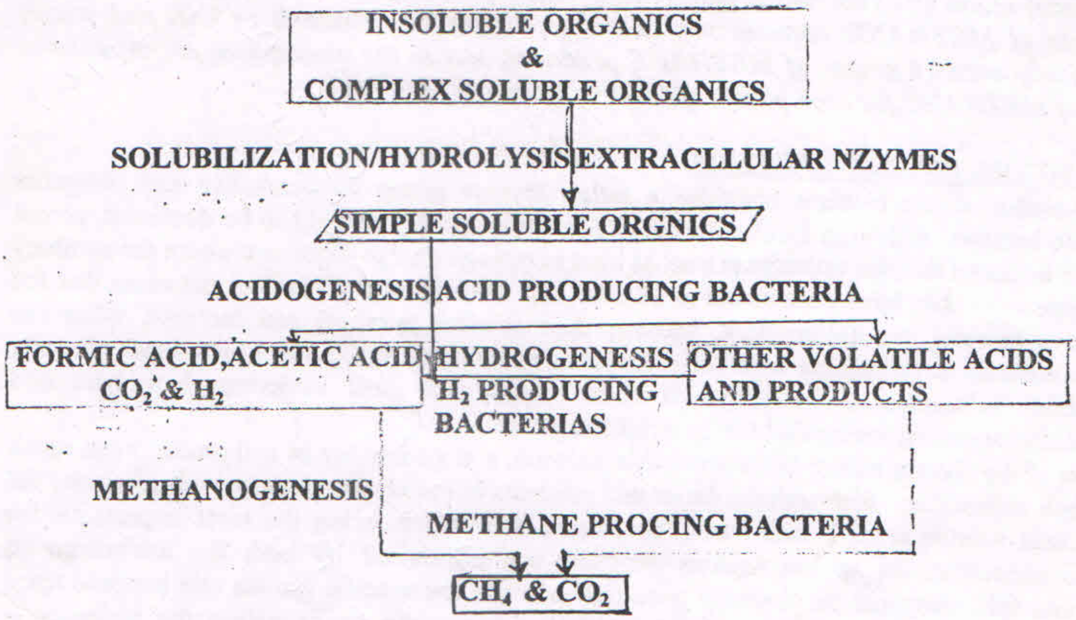

Fig.1 Multi-step nature of anaerobic operations.

Before insoluble organics can be consumed, they must first be solubilized. In addition, large soluble molecules must be reduced in size to facilitate transport across the cell membrane. The reactions responsible for solubilization and size reduction are usually hydrolytic and are catalyzed by enzymes which have been released to the medium by the bacteria. The small molecules resulting from hydrolysis are used as Carbon and energy sources by bacteria which carry out fermentations. The oxidized end products of those fermentations are primarily short-chain volatile acids such as **acetic, propionic, butyric and valeric.** Their production is referred to as ***aciogenesis*** and the responsible organisms are called acid-producing bacteria. Some of the acid producing bacteria produce few reduced organic end products. In addition, some of them utilize volatile acids larger than acetic, as well as reduced organic compounds released by other bacteria, to produce acetic acid, CO_2 and H_2. The collective activity of these hydrogen producing bacteria is called ***hydrogenesis***. Actually, the distinction between id-producing ad hydrogen-producing bacteria is not clear. Since hydrogen-producing bacteria usually produce acids, but acid-bacteria do not at all produce hydrogen, it is probably best to think of the hydrogen-producing bacteria as a subset of the acid-producing group. The combined groups of acid and hydrogen producing bacteria are generally referred to as non-methanogenic bacteria nd their integrated metabolism results primarily in formic acid, acetic acid, CO_2 and H_2. If no hydrogen is formed, the non-methanogenic phase results in insignificant reduction in **Chemical Oxygen Demand (COD).**

The products of the non-methanogenic phase (i.e. formic acid, acetic acid, CO_2 AND H_2.) are utilized by methanogenic bacteria to produce **METHANE GAS.** Although, it is also possible that methane producing bacteria exist, which have the ability to use other volatile acids and organic end products to form **METHANE,** a few of them have been isolated. Consequently that route to **METHANE** is shown as a dotted line in fig.1. With the exception of losses due to microbial inefficiency, almost all of the energy removed from the liquid, is recovered in **METHANE.**

One mole of METHANE requires two moles of oxygen to oxidize it to CO_2 and water, consequently each 16 grams of METHANE produced, lost to the atmosphere corresponds to 5.62 ft^3 of METHANE for each pound of COD stabilized (0.34 m^3/ kg).

NON – METHANOGENIC BACTERIA

The no-methanogenic bacteria comprise a rather diverse group of facultative and obligated anaerobic bacteria. Although facultative bacteria were originally thought to be dominant, recent evidence indicates that the opposite is true, at least in sewage sludge digesters, where the numbers of obligate anaerobes have been found to be over 100 times greater. This does not mean that the facultative bacteria are unimportant, because their relative numbers can increase, when the influent contains large number of them, r when the reactor is subjected to ***shock loads*** of easily fermentable substrates. Nevertheless, it does appear that most important hydrolytic and fermentative reactions are carried out by strict anaerobes.

Because of the diverse nature of the anaerobic bacteria, a large number of end products can result from their metabolism. Although the major end products of the non-methanogenic bacteria are the short chain volatile acids (with acetic, propionic, and butyric being the most important), the relative concentrations of the various products are influenced by both the environmental conditions (pH, temperature, electrode potential etc.) and the specific growth rate imposed upon the culture. One of the most important internal changes can occur within the hydrogen – producing bacteria. If they are not producing, they must use organic compounds as acceptors for the electrons removed, during biological oxidation, resulting in the formation of reduced products such as butanol, ethanol etc. If on the other hand, a hydrogenous enzyme system is active, the electrons may be transferred to hydrogen ions, forming molecular hydrogen (H_2).

$$2e^- + 2H^+ \rightarrow H_2$$

When organisms with active hydrogenous system are present, the production of acetate is maximized while the production of reduced end products is minimized. The exact quantitative contribution of hydrogenesis to anaerobic operations has not yet been determined, because it has only recently been recognized that the hydrogenous enzyme system in some organisms is under very strict metabolic control by molecular hydrogen. For example, *selenomonas ruminatium* will produce only trace amounts of hydrogen in batch culture, if the gas is allowed to accumulate, but will produce much more, if it is continually removed. However, because hydrogen is continuously removed from operating digesters by the methanogenic culture, thereby maintaining its partial pressure at a low level, it is likely that a higher percentage of the non-methanogenic bacteria are capable of producing hydrogen in such reactors. This could help explain why acetate is the major organic end product of the non-methanogenic bacteria in mixed culture.

METHANOGENIC BACTERIA

The exact nature of the bacteria responsible for the production of methane in anaerobic operations is not yet certain, although it appears that two distinct groups are involved. One group obtains its energy from the oxidation of molecular hydrogen, whereas the other group oxidizes acetate.

For many years, the only methanogenic bacteria which had been isolated in pure culture were those which oxidize hydrogen. They are all strictly obligate anaerobes, which obtain their energy from the oxidation and their Carbon from carbon-dioxide. Their cell yield is low. During their metabolism, they also use carbon-dioxide as the terminal electron (hydrogen) acceptor, forming METHANE gas in the process :

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

Several of these species can utilize formic acid as the sole substrate, but this may be because it breaks own easily to yield hydrogen and carbon-dioxide. They are very sensitive to pH, however it appears to be inhibited by values outside the range 6.7 – 7.4.

Most information about the group which oxidizes acetate, has been obtained with enrichment, rather than pure cultures. The one organism capable of utilizing acetate which had been isolated in pure culture was a **methanosarcina**, but its rates were very slow.

Although it has long been known that acetate is a major precursor of METHANE in anaerobic operations, the relative importance of the two possible groups of methanogenic bacteria in its utilization is unknown. The fact that most hydrogen utilizing methanogenic bacteria cannot use acetate for an energy substrate does not mean that they cannot use it to form MTHANE because they have been observed to use it as a terminal electron acceptor. The proposed reaction is :

$$CH_3COOH + 4H_2 \rightarrow 2CH_4 + 2H_2O$$

Thus acetate could serve as a precursor for METHANE as the methanogenic bacteria remove the hydrogen formed by the non-methanogenic bacteria, thereby preventing the accumulation of hydrogen with its resultant inhibition of the hydrogen forming bacteria. The role of the hydrogen utilizing methanogenic bacteria in an enrichment culture degrading propionate has been shown to be as a sink for the hydrogen formed, thereby allowing propionate degradation to continue. Thus they probably play a similar role in anaerobic operations, thereby removing acetate in the process. Acetate utilizing bacteria may play the major role in METHANE formation by mixed cultures degrading complex substrates. However, since radioactive labeling experiments have indicated that even though a portion of the METHANE gas was formed by oxidation of hydrogen, the majority was formed by cleavage of acetate :

$$CH_3COOH \rightarrow CH_4 + CO_2$$

(End of Lecture-I)