WATER POLLUTION AND ANALYSIS CONTINUED

Special Precautions for Wastewater Sampling

• A clean pair of new, non-powdered, disposable gloves will be worn each time a different location is sampled and the gloves should be donned immediately prior to sampling. The gloves should not come in contact with the media being sampled and should be changed any time during sample collection when their cleanliness is compromised.

• Sample containers for samples suspected of containing high concentrations of contaminants shall be stored separately.

• Sample collection activities shall proceed progressively from the least suspected contaminated area to the most suspected contaminated area. Samples of waste or highly contaminated media must not be placed in the same ice chest as environmental (i.e., containing low contaminant levels) or background/control samples.

• If possible, one member of the field sampling team should take all the notes and photographs, fill out tags, etc., while the other members collect the samples.

Sample Handling and Preservation Requirements

1. Wastewater samples will typically be collected either by directly filling the sample container or by using an automatic sampler or other device.

2. During sample collection, if transferring the sample from a collection device, make sure that the device does not come in contact with the sample containers.

Site selection for waste water sampling

Influent

Influent wastewaters are preferably sampled at locations of highly turbulent flow in order to ensure good mixing; however, in many instances the most desirable location is not accessible. Preferable influent wastewater sampling locations include: 1) the upflow siphon following a comminutor (in absence of grit chamber); 2) the upflow distribution box following pumping from main plant wet well; 3) aerated grit chamber; 4) flume throat; 5) pump wet well when the pump is operating; or 6) downstream of preliminary screening.

Effluent

Effluent samples should be collected at the site specified in the permit, or if no site is specified in the permit, at the most representative site downstream from all entering wastewater streams prior to discharge into the receiving waters.

Sample Types

Grab Samples

Grab samples consist of either a single discrete sample or individual samples collected over a period of time not to exceed 15 minutes. The grab sample should be representative of the wastewater conditions at the time of sample collection. The sample volume depends on the type and number of analyses to be performed.

Composite Samples

Composite samples are collected over time, either by continuous sampling or by mixing discrete samples. A composite sample represents the average wastewater characteristics during the compositing period

Characteristics of waste water

Physical Characteristics

The physical characteristics of wastewater include those items that can be detected using the physical senses. They are temperature, color, odor, Dissolved oxygen, Insoluble substances (settleable solids, suspended solids),and foamability.

Dissolved Oxygen

The measurement of DO gives the ready assessment of purity of water.The determination of DO is the basis for BOD test which is commonly used to evaluate the pollution strength of waste waters.

Chemical Characteristics

The chemical characteristics of wastewater of special concern are pH, acidity or alkalinity,COD, Hardness, total carbon,chlorine demand, known organic and inorganic compounds, hydrocarbons,oils,greases etc

Oxygen Demand

It is the amount of oxygen used by bacteria and other wastewater organisms as they feed upon the organic solids in the wastewater.

COD

By definition the COD is the amount of oxygen required to stabilized the organic matter chemically, i.e. the COD is used as a measure of the oxygen equivalent of the organic matter contents of a sample that is susceptible to oxidation by a strong chemical oxidant.

Biological Characteristics of Wastewater

BOD

is defined as the amount of oxygen required by the bacteria while stabilizing decomposable organic matter under aerobic condition. It is written as by BOD or BOD520. "It is the amount of oxygen required by aerobic bacteria to decompose/stabilized the organic matter at a standard temperature of 20oC for a period of 05 days".

The three biological organisms present in wastewater are bacteria, viruses, and parasites.

Bacteria

Sewage consists of vast quantities of bacteria, most of which are harmless to man. However, pathogenic (disease-causing) organisms such as typhoid, dysentery, and other intestinal disorders may be present in wastewater. The bacteria in raw sewage may be expected to in the range from 500, 000 to 5,000,000 per mL. These bacteria are responsible for the decomposition of complex compounds to stable compounds with the help of some extracellular and intracellular enzymes. Depending upon the mode of action of bacteria may be divided into the following three categories;

- Aerobic Bacteria
- Anaerobic Bacteria
- Facultative Bacteria

Determination of BOD

The BOD test takes 5 days to complete and is performed using a dissolved oxygen test kit. The BOD level is determined by comparing the DO level of a water sample taken immediately with the DO level of a water sample that has been incubated in a dark location for 5 days. The difference between the two DO levels represents the amount of oxygen required for the decomposition of any organic material in the sample and is a good approximation of the BOD level.

1. Take 2 samples of water

2. Record the DO level (ppm) of one immediately using the method described in the dissolved oxygen test.

3. Place the second water sample in an incubator in complete darkness at 20o C for 5 days. If you don't have an incubator, wrap the water sample bottle in aluminum foil or black electrical tape and store in a dark place at room temperature (20o C or 68 °F).

4. After 5 days, take another dissolved oxygen reading (ppm) using the dissolved oxygen test kit.

5. Subtract the Day 5 reading from the Day 1 reading to determine the BOD level. Record your final BOD result in ppm

<u>Determination of dissolved oxygen</u>

Steps in the Winkler method of oxygen determination.

1. Manganese(II) ions liberated from the manganese sulfate are loosely bound with excess hydroxide.

$$Mn^{2+} + 2OH^{-} \longrightarrow Mn(OH_2)$$

2. Manganese(II) is oxidized to Manganese(III) in the presence of a strong base and binds the dissolved oxygen.

$$2Mn(OH_2) + \frac{1}{2}O_2 + H_2O \longrightarrow 2Mn(OH_3)$$

3. Free iodine is produced upon acidification of the sample at a rate of one I_2 molecule for each atom of oxygen.

$$2Mn(OH_3) + 2I^{-} + 6H^{+} \longrightarrow 2Mn^{2+} + I_2 + 6H_2O$$

4. Free iodine complexes with excess iodide ions.

$$I2 + I^{-} \longrightarrow I_3^{-}$$

5. The iodine/iodide complex is reduced to iodide with thiosulfate.

$$I_3^{-} + 2S_2O_3^{2-} \longrightarrow 3I^{-} + S_4O_6^{2-}$$

<u>BACTERIAL EXAMINATION OF WATER</u>

The bacteriological examination of water is performed routinely by water utilities and many governmental agencies to ensure a safe supply of water for drinking, bathing, swimming and other domestic and industrial uses. The examination is intended to identify water sources which have been contaminated with potential disease-causing microorganisms. Such contamination generally occurs either directly by human or animal feces, or indirectly through improperly treated sewage or improperly functioning sewage treatment systems. The organisms of prime concern are the intestinal pathogens, particularly those that cause typhoid fever and bacillary dysentery.

Since human fecal pathogens vary in kind (viruses, bacteria, protozoa) and in number, it would be impossible to test each water sample for each pathogen. Instead, it is much easier to test for the presence of nonpathogenic intestinal organisms such as E. coli. E. coli is a normal inhabitant of the intestinal tract and is not normally found in fresh water. Therefore, if it is detected in water, it can be assumed that there has been fecal contamination of the water. In order to determine whether water has been contaminated by fecal material, a series of tests are used to demonstrate the presence or absence of coliforms. The coliform group is comprised of Gram-negative, nonspore-forming, aerobic to facultatively anaerobic rods, which ferment lactose to acid and gas. Two organisms in this group include E. coli and Enterobacter aerogenes; however, the only true fecal coliform is E. coli, which is found only in fecal material from warm-blooded animals. The presence of this organism in a water supply is evidence of recent fecal contamination and is sufficient to order the water supply closed until tests no longer detect E. coli.

STANDARD WATER ANALYSIS

The Presumptive Test

In the presumptive test, a series of lactose broth tubes are inoculated with measured amounts of the water sample to be tested. The series of tubes may consist of three or four groups of three, five or more tubes. The more tubes utilized, the more sensitive the test. Gas production in any one of the tubes is presumptive evidence of the presence of coliforms. The most probable number (MPN) of coliforms in 100 ml of the water sample can be estimated by the number of positive tubes

The Confirmed Test

If any of the tubes inoculated with the water sample produce gas, the water is presumed to be unsafe. However, it is possible that the formation of gas may not be due to the presence of coliforms. In order the confirm the presence of coliforms, it is necessary to inoculate EMB (eosin methylene blue) agar plates from a positive presumptive tube. The methylene blue in EMB agar inhibits Gram positive organisms and allows the Gram-negative coliforms to grow. Coliforms produce colonies with dark centers. E. coli and E. aerogenes can be distinguished from one another by the size and color of the colonies. E. coli colonies are small and have a green metallic sheen, whereas E. aerogenes forms large pinkish colonies. If only E. coli or if both E. coli and E. aerogenes appear on the EMB plate, the test is considered positive. If only E. aerogenes appears on the EMB plate, the test is considered negative. The reasons for these interpretations are that, as previously stated, E. coli is an indicator of fecal contamination, since it is not normally found in water or soil, whereas E. aerogenes is widely distributed in nature outside of the intestinal tract.

The Completed Test

The completed test is made using the organisms which grew on the confirmed test media. These organisms are used to inoculate a nutrient agar slant and a tube of lactose broth. After 24 hours at 37°C, the lactose broth is checked for the production of gas, and a Gram stain is made from organisms on the nutrient agar slant. If the organism is a Gram-negative, nonspore-forming rod and produces gas in the lactose tube, then it is positive that coliforms are present in the water sample.