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# Water and Wastewater Lab manual

Draft Version

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# 1. WATER ANALYSIS

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All waters that occur in nature contain impurities.

The types of constituents and their concentrations vary greatly depending on:
- Natural phenomena
- Human activities

Water quality is described by a series of water quality parameters

A water quality parameter refers to a property of water (such as color, taste, turbidity) or to the composition of water.

The composition of water can be expressed in the concentration of individual compounds or it can be expressed in the concentration of a group of related compounds, such as Biochemical Oxygen Demand (BOD), these are called lump parameters.

Substances present in water are either in solid, liquid, or gas form.

Another distinction can also be made according to particle size (important in water treatment as the required treatment process depends on the size of particles to b e removed; also in water treatment).

There is no universal agreement on particle size distribution. The following classification is an example of particle size distribution.

| **Type of matter** | **Particle size** |
|---|---|
| Suspended matter | > 1 µm |
| Colloidal matter | 0.001 – 1 µm |
| Dissolved (or soluble) | <0.001 µm |

Another example

| **Type of matter** | **Particle size** |
|---|---|
| Suspended matter | > 4.4 µm |
| Colloidal matter | 0.45 – 4.4 µm |
| Dissolved (or soluble) | <0.45 µm |

| | Water Quality Parameters | |
|---|---|---|
| **Physical parameters** | **Chemical parameters** | **Microbiological parameters** |
| TS | COD | coliform |
| VS | BOD | |
| TSS | Nkj-N | |
| pH | NH4-N | |
| Turbidity | NO3 | |
| conductivity | PO4 | |
| | SO4 | |

**Water analysis is essential in:**

- The design and operation of collection, treatment, and reuse facilities
- To asses reactor performance
- To comply with standards
- others

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## 2. BIOCHEMICAL OXYGEN DEMAND (BOD)

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The amount of oxygen required to oxidize a substance to carbon dioxide and water may be calculated by stoichiometry if the chemical composition of the substance is known. This amount of oxygen is known as the theoretical oxygen demand (ThOD).

Organic matter: $C_nH_aO_bN_c$

$C_nH_aO_bN_c+\{n + a/4 –b/2 – 3c/4\}O_2 \rightarrow nCO_2 + \{a/2 – 3c/2\}H_2O + cNH_3$

In contrast to the (ThOD), the COD and (BOD), is a measured quantity that does not depend on knowledge of the chemical composition of the substances in water.

The actual BOD is less than the ThOD due to the incorporation of some of the carbon into new bacterial cells.

**BOD: defined** as the amount of oxygen required to oxidize organic matter by microorganisms under aerobic conditions.

Oxidation only of biodegradable organic matter!

The BOD test is widely used to determine the pollution strength of domestic and industrial wastes in terms of the oxygen that they will require if discharged into natural watercourses in which aerobic conditions exist. The test is one of the most important in stream-pollution-control activities. The test is of prime importance in regulatory work and in studies designed to evaluate the purification capacity of receiving bodies of water.

The BOD test is essentially a bioassay procedure involving the measurement of oxygen consumed by living organisms (mainly bacteria) while utilizing the organic matter present in a waste, under conditions as similar as possible to those that occur in nature. in order to make the test quantitative, the samples must be protected from the air to prevent re-aeration as the dissolved – oxygen level diminishes. In addition, because of the limited solubility of oxygen in water, about 9 mg/l at 20 ºC, strong wastes must be diluted to levels of demand in keeping with this value to ensure that dissolved oxygen will be present throughout the period of the test.

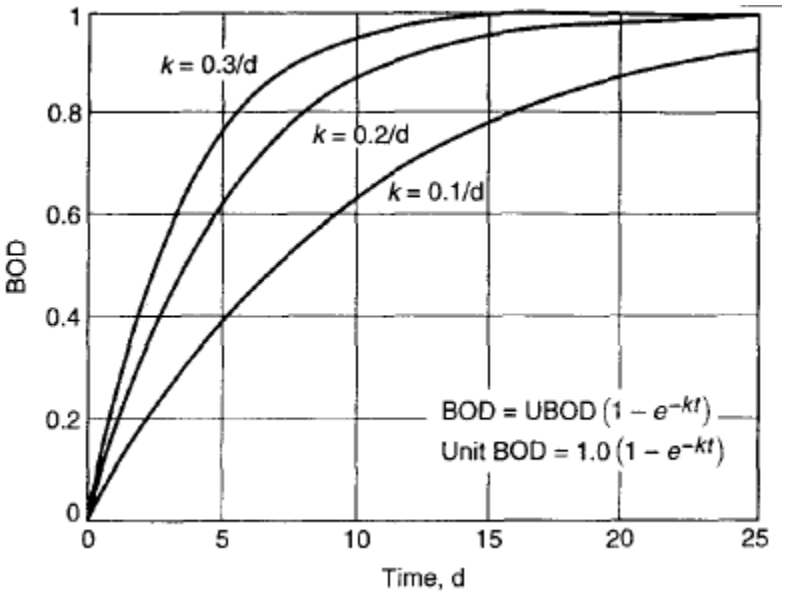
A water samples is inoculated with bacteria that consume the biodegradable organic matter to obtain energy for their life processes. Because the organisms also utilize oxygen in the process of consuming the waste, the process is called aerobic

decomposition. This oxygen consumption is easily measured. The greater the amount of organic matter present, the greater the amount oxygen utilized. **The BOD test is an indirect measurement of organic matter because we actually measure only the change in dissolved oxygen concentration caused by the microorganisms as they degrade the organic matter.**

Although **not all organic matter is biodegradable** and the actual test procedures lack precision, the BOD test is still the most widely used method of measuring organic matter because of the direct conceptual relationship between BOD and oxygen depletion in receiving waters.

**$BOD_5^{20}$**

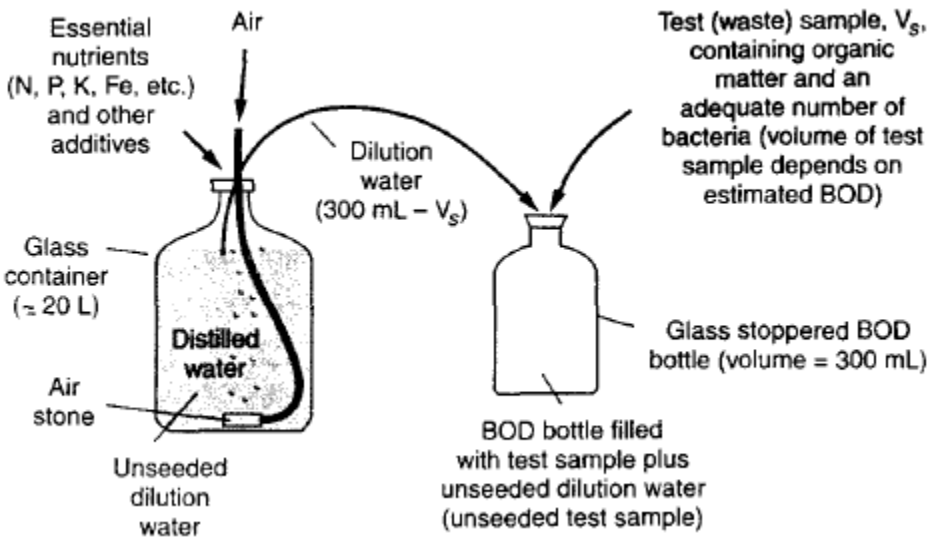
the five-day BOD was chosen as the standard value for most purposes because the test was devised by sanitary engineers in England, where rivers travel times to the sea of less than five days.




**Dilution technique**

Dilutions that result in a residual DO of at least 1 mg/l and a DO uptake of at least 2 mg/l after 5 days incubation produce the most reliable results. Make several dilutions of prepared sample to obtain DO uptake in this range.

DO: dissolved Oxygen




**Calculations:**

$$BOD_5^{20} = \frac{D_0 - D_5}{p}$$

where,

$D_0$: DO of diluted sample immediately after preparation

$D_5$: DO of diluted sample after 5 days incubation at 20 C

P: decimal volumetric fraction of sample used (volume of sample taken/ 300 ml)

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## 3. CHEMICAL OXYGEN DEMAND (COD)

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The COD test is used to measure the oxygen equivalent of the organic material in wastewater that can be oxidized chemically using dichromate in an acid solution.

The Chemical Oxygen Demand (COD) test is widely used as a means of measuring organic strength of domestic and industrial wastes.

The basic principle of the COD test is the oxidation of the organic compounds in the sample by a mixture of potassium dichromate and sulfuric acid, with the use silver ($Ag^{+}$) as a catalyst. The test is conducted at 150 C to accelerate the redox reaction.

The major advantage of the COD test is the short time required.

$$Organic\ \ matter + Cr_2O_7^{2-} + 2H^{+} \rightarrow Cr^{3+} + CO_2 + H_2O$$

$$Cr_2O_7^{2-} : Oxidi\sin g\ \ agent$$

oxidation of organic matter [both biodegradable and non-biodegradable]

COD is very useful to measure the organic matter in toxic wastewaters especially industrial waste

Usually COD = 2 BOD (in raw wastewater)
= 4 BOD (in treated wastewater)

The COD is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong oxidant.

Drawbacks and justifications:

1. The COD test is unable to differentiate between biologically oxidisable and biologically inert organic matter (lignin and glucose)

2. It does not provide any evidence of the rate at which the biologically active material would be stabilized under conditions that exist in nature

3. Inorganic substances that are oxidized by the dichromate increase the apparent organic content of the sample

4. Certain organic substances may be toxic to the microorganisms used in the BOD test.

The major advantage of the COD test is the short time required for evaluation

**COD total and fractions**

The COD test can be performed on different fractions of the sample:

- Raw sample (COD-total)
- Paper filtered sample; pore size 4.4 µm (COD paper filtered)
- Dissolved COD: $COD_{dis}$ <0.45 µm

**CODtotal:** $COD_t$ (Raw sample)

**COD suspended:** $COD_{ss}$

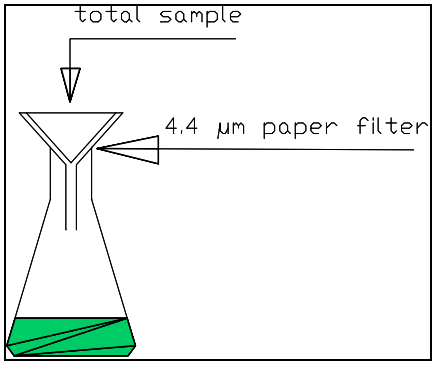
$COD_{ss} = COD_t - COD_{pf}$

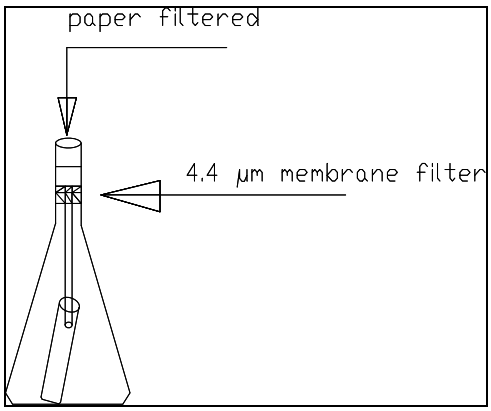
**COD colloidal:** CODcol.

$COD_{col} = COD_{pf} - COD^{mf}$

**COD dissolved:** $COD_{dis}$

$COD_{dis} = COD_{mf}$




paper filtered
4.4 µm membrane filter

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**Micro COD method (photometric)**

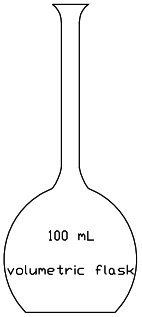
The reduced quantity of potassium dichromate is related to the COD of the sample

Remarks:
The sample should contain less than 1000 mg COD/l. If the sample has a COD higher than 1000 mg/l, dilute with demi water. In this case the dilution factor should be added to the calculation.

**Preparation of standards**

Stock KHP: 850 mg potassium hydrogen phthalate, dried at 120 ºC for 24 hours, is dissolved in 1000 ml H2O. The COD of this stock solution is 1000 mg O2/l

Prepare standard solutions containing a known COD concentration by diluting a known volume, A, of the stock KHP to 100 ml.




| ML/ 100 mL | COD (mg/l) |
|---|---|
| 0 | 0 |
| 2 | 20 |
| 5 | 50 |
| 7 | 70 |
| 10 | 100 |
| 20 | 200 |
| 30 | 300 |
| 40 | 400 |
| 50 | 500 |
| 60 | 600 |
| 70 | 700 |
| 80 | 800 |
| 90 | 900 |

**Procedure**

1. transfer 2.50 ml of standard solution or sample to the digestion tube and add 1.50 ml digestion solution
2. carefully run 3.50 ml $H_2SO_4/Ag_2SO_4$ down inside of tube so an acid layer is formed under the sample – digestion solution layer. Tightly cap tubes and swirl several times to mix carefully; do not invert the tube!
3. place the tubes in the heating block at 150 C for 2 hours.
4. allow them to cool, mix the content and let particles settle
5. the next day: transfer the content gently and without mixing to a 1 cm cell and measure the absorbance at 600 nm against blank
6. plot the absorbance against the known COD in order to get a calibration line.
7. read absorbance of samples and compare to calibration line, determine the mathematical equation of this line.

## 4. pH

Dr. Nidal Mahmoud

pH is a term used rather universally to express the intensity of the acid or alkaline condition of a solution. It is a way of expressing the hydrogen-ion concentration. It is one of the most important environmental parameters. In the field of water supplies, it is a factor that must be considered in chemical coagulation, disinfection and corrosion control. In wastewater treatment employing biological processes, pH must be controlled within a range favorable to the particular organisms involved. Since, the concentration range suitable for the existence of most biological life is quite narrow and critical (typically 6 –9).

Water dissociation: $H_2O \Leftrightarrow H^+ + OH^-$

By substituting into the equilibrium equation, we obtain:

$$k = \frac{[H^+][OH^-]}{[H_2O]}$$

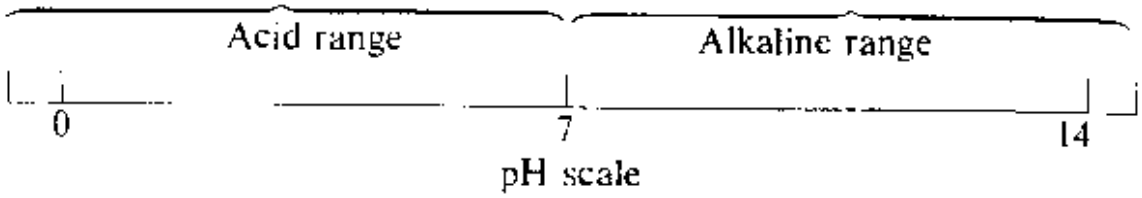
Since the concentration of water is so extremely large and is diminished so very little by the sight degree ionization, it may be considered as constant, and the equilibrium may be written as:

$$k_w = [H^+][OH^-]$$

$k^w = 10^{-14}$

$$pH = -\log[H^+] = \log\frac{1}{[H^+]}$$

The pH scale is usually represented as ranging from 0 to 14, with pH 7 at 25 ˚C representing absolute neutrality.




Because $k_w$ changes with change in temperature, the pH of neutrality changes with temperature as well, being 7.5 at 0 ˚C and 6.5 at 60 ˚C.

Acid conditions increase as pH values decrease, and alkalinity conditions increase as the pH values increase.

## 5. SALINITY, CONDUCTIVITY AND TDS

Dr. Nidal Mahmoud

Salinity of water can be determined by measuring its electrical conductivity. The conductivity of a solution is a measure of its ability to carry an electrical current, and varies both with the number and type of ions the solution contains. Most dissolved inorganic substances in water supplies are in the ionized form (like $Cl^-$, $Na^+$, $Ca^{2+}$ and $SO_4^{2-}$) and so contribute to the specific conductance. The electrical conductivity (EC) of a water is used as a rapid measure replacing total dissolved solids (TDS) concentration. The EC measurements give a practical estimate of the variation in dissolved minerals content of a specific water supply. Also, by the use of an empirical factor, specific conductance can allow a rough estimate to be made of the dissolved mineral content of water samples.

The electrical conductivity can be expressed as microsiemens per centimeter (µS/cm). Values for salinity are also reported as TDS in mg/l. For most agricultural irrigation purposes, the values for EC and TDS are related to each other and can be converted within an accuracy of about 10% using:

TDS (mg/l) ≈ EC (µS/cm) x 0.64

*The used conversion factor of 0.64 can vary in the range (0.55 to 0.7)*

Salinity can also be reported as pats per thousands (‰) [ gram of solids/ kg of solution; gram of solids/ 1000 gram of solution].

Salinity is an important parameter in the analysis of drinking water, irrigation water, industrial water and sea water.

**Health effects**. High levels of total dissolved solids may impart an objectionable taste to drinking water. Chloride, in particular, has a low taste threshold. Sodium sulfate and magnesium sulfate levels above 250 mg/l in drinking water may produce a laxative

effect. Excess sodium may affect those restricted to low sodium diets and pregnant women suffering from toxemia. The limit of TDS in drinking water is less than 500 mg/l.

**Irrigation effects**. Salinity of an irrigation water determined by measuring its electrical conductivity is the most important parameter in determining the suitability of a water for irrigation. The presence of salts affects plant growth in three ways: (**1**) osmotic effects, caused by the total dissolved salt concentration in the soil water; (**2**) specific ion toxicity caused by the concentration of individual ions, and (**3**) soil particle dispersion, caused by high sodium and low salinity. With increasing salt salinity in the root zone, plants expend more of their available energy on adjusting the salt concentration within the tissue (osmotic adjustment) to obtain needed water from the soil. Consequently, less energy is available for plant growth. So high salinity may interfere with the growth of vegetation. Salt may even decrease the osmotic pressure to the degree that it might cause water to flow out of the plant to achieve equilibrium. The influence of the salt on the crops depends on the type of the plant as some plants are less sensitive to high salts concentration (known as salt tolerant). The limit of TDS in irrigation water is in generally in the range 500 – 1000 mg/l (dependent upon crop sensitivity).

**Industrial effects**. Dissolved solids may corrode metallic surfaces. Salt in intake water may interfere with chemical processes within the plant (factory). Also, high salts may affect the taste of beverages. The limit of TDS in industrial water depends on the type of industry, for instance, for the production of fine paper TDS should be less than 200 mg/l; for Groundwood paper less than 850 mg/l. Industry can de-ionize water to meet requirements; economics\ is the limiting factor.

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## 6. TURBIDITY

Dr. Nidal Mahmoud

Turbidity is an indication of the clarity of a water and is defined as the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through a sample of water. So turbidity, a measure of the light-transmitting properties of water. Turbidity is caused by material in suspension. The turbidity test is used to indicate the quality of waste discharges and natural waters with respect to colloidal and residual suspended matter. The measurement of turbidity is based on comparison of the intensity of light scattered by a reference suspension (formazin suspension) under the same conditions (Standard Methods, 1998) and thus produces an equivalent effect. The results of turbidity measurements are reported as nephelometric turbidity units (NTU). Actually, a variety of definitions, methods of measurement, instruments, standards and units of measurement have been used.

Nephelometric method is used to measure turbidity. In this method, light is allowed to strike a suspension at a right angle to photoelectric cell of the instrument. The light reflected by the dispersed particles (Tyndall effect) is recorded. The amount of light scattered depends on the number, size, shape and refractive index of the particles, etc.

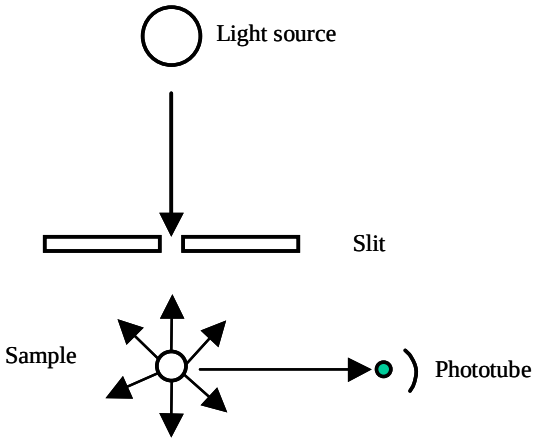


**Fig . Schematic diagram of a nephelometer**

In general, there is no strong relationship between turbidity and the concentration of total suspended solids in untreated wastewater. There is, however, a reasonable relationship between turbidity and total suspended solids for the settled and filtered secondary effluent from the activated sludge process. The general form of the relationship is as follows:

TSS, mg/l ≈ $TSS_f$x T
Where,
TSS = total suspended solids, mg/l
$TSS_f$ = factor used to convert turbidity readings to total suspended solids, (mg/l TSS)/NTU
T = turbidity, NTU
The specific value of the conversion factor will vary for each treatment plant, depending primarily on the operation of the biological treatment process. The conversion factors for settled secondary effluent and for secondary effluent filtered with a granular medium depth filter will typically vary from 2.3 to 2.4 and 1.3 to 1.6, respectively.

One of the problems with the measurement of turbidity (especially low values in filtered effluent) is the high degree of variability observed. Another problem often encountered is the high-absorbing properties of the suspended material.

As a result it is almost impossible to compare turbidity values reported in the literature. However, turbidity readings at a given facility can be used for process control.

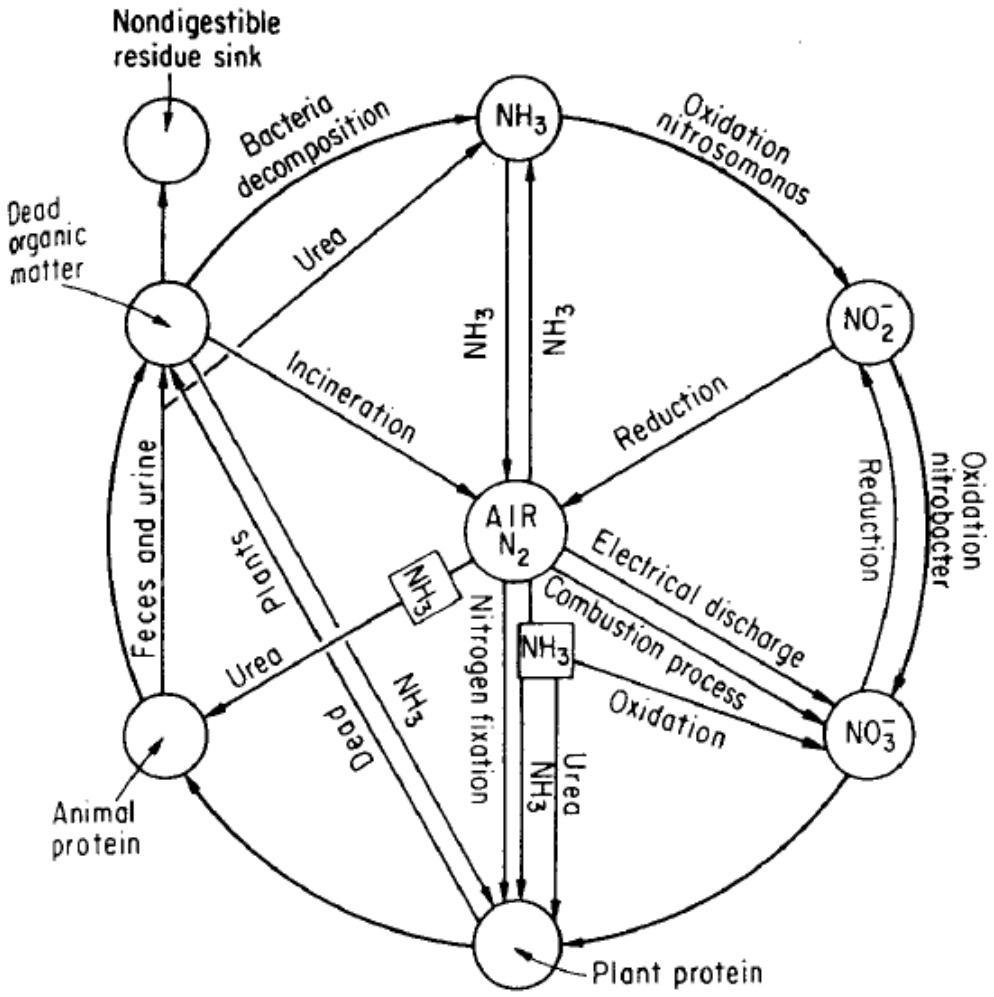
Turbidity measurements are often used to monitor the performance of treatment works processes. Turbidity meters are frequently installed on–line to check the amount of flocculent material being carried over from sedimentation tanks and to check individual filter performance. According to the WHO guidelines for drinking water quality, turbidity should be below 5 NTU, and preferably < 1 NTU to assure efficient disinfections. In excess of 5 NTU turbidity is noticeable in water, consequently objectionable to consumers so aesthetically unattractive, and also may be harmful.

## 7. NITROGEN

Dr. Nidal Mahmoud

The compounds of nitrogen are of great interest to environmental engineers because of the importance of nitrogen compounds in the atmosphere and in the life processes of all plants and animals. Nitrogen is essential to life and is a component of proteins and nucleic acids in microbial, animal, and plants cells. Both nitrogen and phosphorous are essential to the growth of microorganisms (bacteria, algae, .) and plants as such are known as nutrients.

Nitrogen can be found in nature in different forms, i.e. $NH_3$, $NO_2$, $NO_3$ and others. The relationships that exist between the various forms of nitrogen compounds and the changes that can occur in nature are best illustrated by a diagram known as **the nitrogen cycle**.




**The nitrogen cycle**

From the nitrogen cycle, it can be seen that the atmosphere serves as a reservoir from which nitrogen is constantly removed by the action of electrical discharge and nitrogen-fixing bacteria. During electrical storms large amounts of nitrogen are oxidized to $N_2O_5$, and its union with water produces $HNO_3$, which is carried to the earth in the rain. Nitrates are also produced by direct oxidation of nitrogen or of ammonia in the production of commercial fertilizers. The nitrates serve to fertilize plant life and are converted to proteins. Through the **nitrogen assimilation** mechanisms nitrate is taken up by plants and microorganisms and then converted to nitrite and then to ammonium, and ends up as protein.

$$NO_3^- + CO_2 + greenplants + sunlight \rightarrow protein$$

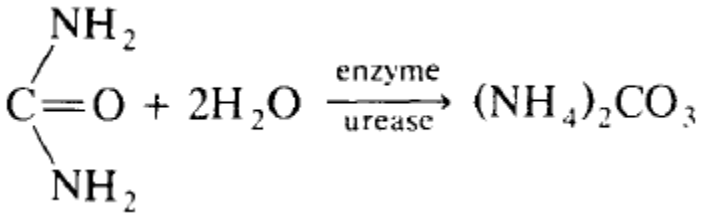
In addition, ammonia and ammonium compounds are applied to soils to supply plants with ammonia for further production of proteins.

$$NH_3 + CO_2 + greenplants + sunlight \rightarrow protein$$

Animals and human beings are incapable of utilizing nitrogen from the atmosphere or from inorganic compounds to produce proteins. They are dependent upon plants, or other animals that feed upon plants, to provide protein. Atmospheric nitrogen is also converted to proteins by "**nitrogen-fixing**" bacteria. **Nitrogen fixation** is driven by an enzyme called nitrogenase.

$$N_2 + \text{special bacteria} \rightarrow protein$$

Within the animal body, proteins are used largely for growth and repair of muscle tissue. Some may be used for energy purposes. In any event, nitrogen compounds are released in the waste products of the body during life. At death the proteins stored in the body become waste matter for disposal. Afterwards, the protein undergoes the process of **nitrogen miniralization or ammonification**, through which organic nitrogen is transformed to inorganic forms ($NH_4^+$). This process is driven by a wide variety of microorganisms, e.g. bacteria, fungi, etc. The urine contains the nitrogen resulting from the metabolic breakdown of proteins. The nitrogen exists in urine principally as urea which is hydrolyzed rather rapidly by the enzyme urease to ammonium carbonate:



The feces of animals contain appreciable amounts of unassimilated protein (organic nitrogen). It and the protein remaining in the bodies of dead animals and plants are converted in large measure to ammonia by the action of hetertrophic bacteria, under aerobic or anaerobic conditions:

$$\text{protein (organic N)} + bacteria \rightarrow NH_3$$

Some nitrogen always remains in nondigestible matter, and as such it becomes part of the residues in nature, e.g. in the sediments.

The ammonia released by bacterial action on urea and proteins may be used by plants directly to produce plant nitrogen. If it is released in excess of plant requirements, the excess is oxidized by autotrophic nitrifying bacteria. This process is called **nitrification**. The *Nitrosomonas* group of nitrifying bacteria, known as the nitrite formers, convert ammonia under aerobic conditions to nitrites and derive energy from the oxidation:

$$2NH_3 + 3O_2 \xrightarrow{bacteria} 2NO_2^- + 2H^+ + 2H_2O$$

The nitrites are oxidized by the *Nitrobacter* group of nitrifying bacteria, which are also called the nitrate formers.

$$2NO_2^- + O_2 \xrightarrow{bacteria} 2NO_3^-$$

The nitrates formed may serve as fertilizer for plants. Nitrates produced in excess of the needs of plant life are carried away in water percolating through the soil because the soil does not have the ability to hold them. This frequently results in relatively high concentrations of nitrates in groundwaters, and is an extensive problem in Gaza and some localities in the West bank.

Under **anaerobic** conditions **nitrates** and **nitrites** are both reduced by a process called **denitrification**. Presumably nitrates are reduced to nitrites, and then reduction of nitrites occur. Nitrites reduction is carried by bacteria to nitrogen gas, which escapes to the atmosphere. This constitutes a serous loss of **fertilizing** matter in soils when anaerobic conditions develop. The formation of nitrogen by reduction of nitrates is sometimes a problem in the activated sludge process of wastewater treatment. Prolonged detention of activated sludge problem in final settling tanks allows formation of sufficient nitrogen gas to buoy the sludge, if nitrates are present in adequate amounts. This is often referred to as the “rising” sludge problem.

Advantage is taken of denitrification in one proposed scheme for removing nitrogen from wastes where this is required to prevent undesirable growths of algae and other aquatic plants receiving waters, and also for the protection of groundwater. Ammonia and organic nitrogen are first biologically converted to nitrites and nitrates by aerobic treatment. The waste is then placed under anoxic conditions, where denitrification converts the nitrites and nitrates to nitrogen gas, which escapes to the atmosphere. For denitrification to occur, organic matter must be present, and is oxidized for energy while nitrogen is being reduced.

**Forms of nitrogen and analysis forms**

**Total nitrogen** consists of organic nitrogen, ammonia, nitrite and nitrate.
**Organic nitrogen**, as Protein
**Ammonia nitrogen** $NH_4^+$ - N
**Nitrite nitrogen** $NO_2^-$ N
**Nitrate nitrogen** $NO_3^-$ N
**Kjeldahl nitrogen**: organic nitrogen and ammonia

The $NH_4^+$ - N, $NO_2^-$ N, and $NO_3^-$ N are inorganic nitrogen

## *7.1 Ammonia Nitrogen $NH_4^+$-N*

### Determination of ammonia nitrogen by Nesslerization method

### Reagents

**a)** **Rochelle salt**: dissolve 50g potassium sodium tartrate tetrahydrate, $KNaC_4H_4O_6.4H_2O$ in 100 mL.

**b)** **Nessler reagent**: dissolve 100 g $HgI_2$ and 70 g KI in a small quantity of water and add this mixture slowly, with stirring, to a cool solution of 160 g NaOH dissolved in 500 mL. Dilute to 1 L. Store in rubber-stoppered borosilicate glassware and out of sunlight to remain reagent stability for up a year under normal laboratory conditions. **Caution: TOXIC – take acre to avoid ingestion**.

**c)** **Stock $NH_4Cl$**: Dissolve 3.819 g anhydrous $NH_4Cl$, dried at 105 ˚C, in water and dilute to 1000 mL. → 1.00 mL = 1.00 mg N

**d)** **Standard $NH_4Cl$**: dilute 10.00 mL stock solution to 1000 mL. → 1.00 mL = 0.01 mg N

## Apparatus

Spectrophotometer for use at 425 nm with 1 cm cells

**Calibration:**

1. Make a series of standards by diluting 0; 0.50; 1.00; 2.00; 3.00; 5.00; 7.00; and 10 mL standard solution to 50.0 mL.
2. Add 2.0 drops Rochelle salt solution and mix.
3. Add 2.0 mL Nessler reagent and mix.
4. Measure the absorbance at 425 nm in a 1 cm cell after 15 minutes against $H_2O$.
5. Determine the mathematical expression for the calibration line (use excel)

## Procedure

1. Take 50.0 mL sample or (a sample portion diluted to 50 mL).
2. Add 2.0 drops Rochelle salt solution and mix.
3. add 2.0 mL Nessler reagent and mix
4. measure the absorbance at 425 nm in a 1 cm cell after 15 minutes against $H_2O$.

**Calculation**

$$NH_4 - N\,(mg/L) = \frac{mg\,NH_4 - N\,in\,50mL}{mL\ sample} x1000$$

### *7.2 Kjeldahl nitrogen*

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## 8. PHOSPHATE

Dr. Nidal Mahmoud

Phosphate is the most significant inorganic phosphorous compound in engineering practice. The organically bound phosphorous is usually of a minor consideration.

All surface water supplies support growth of minute aquatic organisms. The free swimming and floating organisms are called plankton and are of great interest to environmental engineers. The plankton are composed of animals, zooplankton, and plants, phytoplankton. The latter are predominantly algae and cyanobacteria, and since they are chlorophyll – bearing organisms, their growth is influenced greatly by the amount of fertilizing elements in the water, i.e. nitrogen and phosphorous. The limitation in the amounts of these elements is usually the factor that controls their rate of growth. Where both nitrogen and phosphorous are plentiful, algal blooms occur which may produce a variety of nuisance conditions.

Domestic wastewater is relatively rich in phosphorous compounds. Most of the inorganic phosphorous are contributed by human wastes as a result of the metabolic breakdown of protein and elimination of the liberated phosphorous in the urine. The amount of phosphorous released is a function of protein intake and detergents used and, for the average person in Palestine, this release is considered to be 0.9 – 1.6 g /day, and in the United States 1.5 g/day.

Most heavy-duty synthetic detergent formulations designed for the household market contains large amounts of polyphosphate. The use of these materials as a substitute for soap has greatly increased the phosphorous content of domestic wastewater. It has been estimated from sales of polyphosphate to the detergent industry that domestic wastewater probably contains from two to three times as much inorganic phosphorous at the present time as it did before synthetic detergents became widely used, unless local ordinances limit the use of phosphate-based detergents.

### Apparatus

a) spectrophotometer for use at 880 nm with 1 cm cell.
b) acid-washed glassware: phosphate contamination is common because of its absorption on glass surfaces. Clean all glassware with diluted HCL and rinse well with water (avoid using commercial detergents containing phosphate).

### Reagents

a) $H_2SO_4$ 2.5 M: fill a flask with 420 mL $H_2O$ and add 70 mL concentrated $H_2SO_4$.
b) Potassium antimonyl tartrate: dissolve 1.3715 g $K(SbO)C_4H_4O_6.1/2H_2O$ in 400 mL $H_2O$ and dilute to 500 mL in a volumetric flask. Store in a glass stoppered bottle.
c) Ammonium heptamolybdate: dissolve 20 g $(NH_4)6Mo_7O_{24}.4H_2O$ in 500 mL $H_2O$. Store in a glass stoppered bottle.
d) Ascorbic acid 0.1 M: dissolve 1.76 g ascorbic acid in 100 mL $H_2O$. This solution is stable for about 1 week at 4 ºC.
e) Combined reagent: mix the above reagents in the following order:
   125 mL 2.5M $H_2SO_4$
   + 12.5 mL $K(SbO)C_4H_4O_6.1/2H_2O$
   + 37.5 mL $(NH_4)6Mo_7O_{24}.4H_2O$
   + 75 mL ascorbic acid

   mix after addition of each reagent and let reagents reach room temperature before they are mixed. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before the next solution is added. This reagent is only stable for *4 hours*.

f) Stock P solution: dissolve 439 mg $KH_2PO_4$ (dried at 105 ºC for a few hours) in 1000 mL
   1 mL = 100 µg $PO_4^{3-}$-P
g) standard P solution: dilute 5 mL stock P to 100 mL
   1 mL = 5 µg $PO_4^{3-}$-P
h) Phenolphthaleine indicator: dissolve 0.5 g in 50 mL ethanol and add 50 mL $H_2O$

**Calibration**

1) transfer the following amounts of standard P to 100 mL volumetric flasks: 0; 2; 3 ; 5; 10 and 20 mL
2) add 16 mL combined reagent, fill up to the mark and mix
3) measure the absorbance between 10 – 30 min at 880 nm with a 1 cm cell against $H_2O$
4) plot the absorbance against µg P/100mL and determine the mathematical expression of the calibration line.

Procedure for ortho- $PO_4^{3-}$

1. pipet 50 mL 0.45 µm filtered sample or less to a 100 mL volumetric flask, add 1 drop phenolphthaleine indicator, if a red colour develops (pH>8.3) add drop-wise 2.5 M $H_2SO_4$ to just discharge the colour.
2. add 16 mL combined reagent, fill up to the mark and mix.
3. measure the absorbance at 880 nm between 10 – 30 min with a 1 cm cell.

$$mgP/l = \frac{\mu gP \text{ in } 100 \text{ ml end volume}}{\text{ml sample}}$$

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## 9. SULPHATE

Dr. Nidal Mahmoud

Sulphur is an abundantly available element in nature, and particularly in seawater which represents the largest reservoir of sulphate. Other sources in the environment also include sulphur-containing minerals, e.g. pyrite ($FeS_2$), fossil fuels and organic matter. It is an essential element for micro-organisms as it enters into the composition of the amino acids which are the building block of protein.

Waters containing high concentrations of sulphate, caused by the leaching of natural deposits may be undesirable as drinking waters because of their laxative effects.

High sulphate concentrations as well as low pH conditions can result in streams that are fed by drainage from abandoned coal mines. The sulphide minerals present are oxidised through a combination of bacterial and chemical action as follow:

$$2FeS_2(pyrite) + 7O_2 + 2H_2O \overset{bacteria}{\rightarrow} 2Fe^{2+} + 4SO_4^{2-} + 4H^+$$

The major problem associated with the anaerobic treatment of sulphate-rich wastewater (municipal and industrial) is the production of sulphide. Since sulphide can lead to several problems such as toxicity, bad smell, corrosion, deteriorated quantity and quality of the biogas and reduction of the COD removal efficiency.

$$SO_4^{2-} + \text{organic matter} \overset{anaerobic}{\underset{bacteria}{\rightarrow}} S^{2-} + H_2O + CO_2$$

$$S^{2-} + H^+ \Leftrightarrow HS^-$$

$$HS^- + H^+ \Leftrightarrow H_2S$$

Moreover, the high sulphate content in a wastewater is harmful to the sewerage system. As $H_2S$ gas may be oxidised to $H_2SO_4$ that causes damages to cement containing constructions such as manholes and pipes.

$$H_2S + 2O_2 \overset{aerobic}{\underset{bacteria}{\rightarrow}} H_2SO_4$$

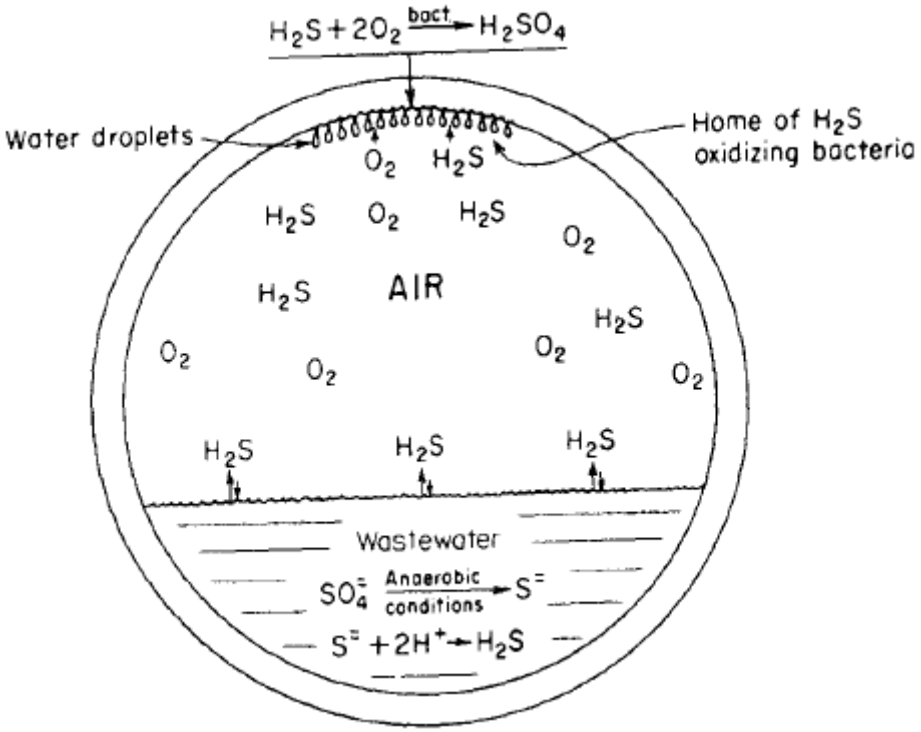


Fig. 1. Formation of hydrogen sulphide in sewers and "crown" corrosion resulting from oxidation of hydrogen sulphide to sulphuric acid

## Principle

$SO_4^{2-}$ is measured colorimetrically using the turbidimetric method. $SO_4^{2-}$ is precipitated in an acetic acid medium with $BaCl_2$ so as to form $BaSO_4$ crystals of uniform size.

## Apparatus

a) Magnetic stirrer with constant stirring speeds; use magnets of identical shape and size
b) Spectrophotometer for use at 420 nm
c) Stop watch
d) Measuring spoon, capacity 0.20 – 0.30 mL

## Reagents

a) Buffer A: dissolve 30 mg $MgCl_2.6H_2O$, 5 g $CH_3COONa.3H_20$, 1 g $KNO_3$ and 20 mL $CH_3COOH$ in 500 mL $H_2O$ and make up to 1000 mL.
b) $BaCl_2$ crystals
c) Stock $SO_4^{2-}$: 1 ml = 1 mg $SO_4^{2-}$

d) Standard $SO_4^{2-}$: dilute 100 mL stock to 1000 mL ➔ 1 mL = 100 µg $SO_4^{2-}$

**Calibration**

1) Pipet 0; 10; 15; 20; 25; 30; 35 and 40 ml standard in a 100 ml volumetric flask and fill up to the mark. This covers the range of 0 – 4000 µg $SO_4^{2-}$ / 100mL
2) Pour content of volumetric flask in a 250 mL beaker + magnet; add 20 ml buffer and mix
3) While stirring, add a spoonful of $BaCl_2$ and begin timing immediately
4) Stir for 60 ±2 sec at constant speed
5) After stirring period has ended, pour solution into the absorption cell and measure the absorbance at 420 nm at 5 ± 0.5 minute
6) Plot absorbance against µg $SO_4^{2-}$ / 100mL

**Procedure** for $SO_4^{2-}$ concentration between 10 – 40 mg/L

1. Measure 100 mL sample, or a suitable portion diluted to 100 mL in a volumetric flask, into a 250 mL beaker + magnet, add 20 mL buffer and mix
2. Proceed as mentioned in the calibration procedure from step 3 on.

**Calculations**

$$mgSO_4^{2-}/l = \frac{\mu gSO_4^{2-} \text{ in 100 ml end volume}}{\text{ml sample}}$$

## 10. COLIFORM

Dr. Nidal Mahmoud

Many bacteria are found in water. Most of them are of no sanitary significance, some are indicators of pollution but are harmless; others, few in numbers, are pathogenic. These include bacteria causing typhoid fever, paratyphoid, dysentery, and cholera.

Bacterial populations are a natural component of lakes, rivers and streams. These bacteria are numerous and diverse assemblage of organisms. The immense numbers of these small organisms can have an enormous impact on processes that occur in aquatic ecosystems such as carbon, nitrogen, and sulfur transformations. They can also have an impact on the quality of water by controlling the amount of oxygen in the water and causing diseases in aquatic organisms as well as humans. The **bacteria that can cause disease are called pathogenic bacteria**.

The majority of microorganisms that cause diseases in humans originate directly from human sources. Much of human contamination can be traced to leaking and overflowing sanitary sewer systems, wastewater treatment facilities, leachate from septic tanks, and fecal matter associated with storm runoff from areas with high densities of wildlife, pets, or livestock. Pathogenic bacteria found in wastewater may be discharged by human beings who are infected with disease or who are carriers of a particular disease.

Long before modern methods for testing water and isolating bacteria had been developed, sewage was recognized as a source of pathogenic bacteria. It has been known since the middle ages that water contaminated with sewage could cause disease. After that, in 1885, it was discovered that a particular type of bacteria called **coliform bacteria** were **numerous** and could always be detected in animal feces and sewage. Although coliform bacteria were not known to cause any illness, their presence was thought to be **a predictor of other disease-causing agents**, i.e. **bacteria**, **protozoan**, **viruses**, and this finding established coliform bacteria as a "**microbial indicator**" of sewage contamination.

Groundwater normally do not contain many bacteria since the effects of filtration, exposure to unfavorable environment, and time will eliminate most of them, including those of sanitary significance. Some shallow wells may contain considerable numbers, but these are frequently due to lack of safeguards in well construction. Soils having cracks may allow insufficiently filtered waters to enter wells or springs. The water of deep wells may have very few bacteria.

Untreated surface waters contain many bacteria. The sanitary engineer is not concerned with most of them. The coliform group is of great importance and includes a number of organisms, e.g. *Aerobacter aerogenes*, *Aerobacter cloaceae*, and *Escherichia coli* (*E. Coli*). *Aerobacter aerogenes* is widely distributed in nature, and normally found on plants and grains, in the soil, and to a varying degree in the feces of man and animals. *Aerobacter cloaceae* is found in the feces of man and animals, and also in soils. *Escherichia coli* (*E. Coli*) normally inhibits in the intestinal tract of man and animals and is excreted with the feces.

The coliforms, therefore, are useful as indicators of pollution, since they show that the water has been in contact with soils or plants or has been polluted by sewage so recently that the bacteria have not died out naturally or been removed by natural filtration or artificial treatment.

Coliforms are of importance not only because they indicate pollution but also because their absence or presence, and their number, can be determined by routine laboratory tests. Tests for pathogens are not adapted to such routine work and are made only in special investigations. **The difficulties with routine testing of pathogens in water are due to the following reasons:**

- Present in low numbers
- Limited survival time
- Numerous pathogens to analyze
- Time and cost prohibitive

**Total coliform bacteria:**

- Sources: fecal material (Inhabit the intestinal tract of animals), soil, water, grain
- Some capable of reproduction in the environment

**Fecal coliform bacteria:**

- Subset of the total coliform group
- Separated from non-fecal coliforms by growth at 44.5 ºC
- Sources: fecal material (from warm blooded animals)
- Capable of limited survival and growth in the environment
- Primary example is *Escherichia coli* (*E. coli*)

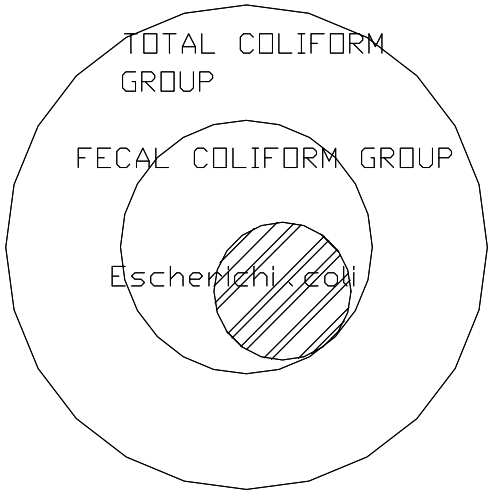


Fig. 1. Simplified classification of the coliform bacteria group

The preferred method for bacterial enumeration is the *membrane filter technique.* In this procedure a measured volume of water is drawn through a cellulose acetate or glass filter with openings less than 0.50 µm. The bacteria present in the sample will be retained upon the filter. The filter is rinsed with a sterile buffer solution, placed upon a pad saturated with a suitable nutrient medium, and incubated at an appropriate temperature. The bacteria which are able to **grow upon the nutrient medium** will produce **visible colonies** which can be counted, each colony representing one bacterium in the original sample. If the sample contains too many bacteria the filter may be overgrown, and counting impossible. In such circumstances smaller samples or diluted samples are used. The routine membrane test provides special procedures which permit separation of **coliforms** of **fecal** and **non-fecal** origin.

The medium used and the temperature of incubation depend upon the bacteria which are being enumerated. For coliform bacteria M-Endo Broth is used with incubation at 35 ºC for 20 to 22 hours. **Coliform bacteria** will produce **colonies** which are pink to dark red with a **golden metallic sheen**, often with a **greenish tent**. Noncoliform bacteria which grow upon this medium will lack the characteristics sheen.

**Fecal coliforms** are assumed to be acclimated to a temperature corresponding to that in the intestines, while nonfecal coliforms would find such temperatures lethal or, at least, inhibiting. Membrane filter analysis for fecal coliforms is conducted at a temperature of 44.5 ºC for 22 hours. On the medium ordinarily used (**M-FC Broth**) fecal coliform colonies are blue. Other bacteria which grow upon this medium are gray to cream colored.

**Procedure**

**Fecal coliform**

**Preparation M FC broth Base**

1. Suspend 3.7 g in 100 mL distilled or deionised water
2. Add 1 mL 1% Bacto Rosolic Acid in 0.2N NaOH solution and heat to boiling
3. Cool to room temperature and add 2 mL of broth to each sterile absorbent pad placed in a Petri dish. The Petri dish should be labeled.

**Test:**

1. Place membrane filter (0.45 µm) through which the sample (100 mL) has been filtered onto the top of the saturated absorbent pad.
2. Place cover of Petri dish on tightly, and incubate at 44.5 ºC for 22 – 24 hours
3. Count

**Total coliform**

**Preparation M Endo broth Base**

1. Suspend 4.8 g in 100 mL distilled or deionised water containing 2 mL of ethanol
2. Heat to boiling; let it cool to room temperature.
3. Dispense onto sterile absorbent pads.

**Test:**

1. Place membrane filter (0.45 µm) through which the sample (100 mL) has been filtered onto the top of the saturated absorbent pad.
2. Place cover of Petri dish on tightly, and incubate at 35 ºC for 24 ± 2 hours
3. Count

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## 11. ALKALINITY

Dr. Nidal Mahmoud

The alkalinity of water is a measure of its capacity to neutralise acid.

The major portion of the alkalinity in natural waters is caused by three major classes of materials which may be ranked in order of their association with high pH values as follows: (1) hydroxide ($OH^-$), (2) carbonate ($CO_3^{2-}$), and (3) bicarbonate ($HCO_3^-$)

Most of the natural alkalinity in waters is due to ($HCO_3^-$) produced by the action of $CO_2$ and $H_2O$ on limestone:

$$\underset{insoluble}{CaCO_3} + H_2O + CO_2 \overset{from(soil)^+}{\underset{bacteria}{\Leftrightarrow}} \Leftrightarrow Ca^{2+} + 2HCO_3^-$$

$CO_2$ originates from bacterial decomposition of organic matter.

Alkalinity is normally divided caustic or phenolphthalein alkalinity above pH 8.3; and total alkalinity above pH 4.5

Total alkalinity = $[OH^{-1}] + [CO_3^{2-}] + [HCO_3^-]$

**Hydroxide, Carbonate, and Bicarbonate Alkalinity**

A graphical representation of typical titration obtained with the various combinations of alkalinity is shown in the following figure

**Hydroxide only.** Samples containing only hydroxide alkalinity have a high pH, usually well above 10. Titration is essentially complete at the phenolphthalein end point.

**Carbonate only.** Samples containing only carbonate alkalinity have a pH of 8.5 or higher. The titration to the phenolphthalein end point is exactly equal to one-half of the total titration. In this case carbonate alkalinity is equal to the total alkalinity.

**Hydroxide-carbonate.** Samples containing hydroxide and carbonate alkalinity have a high pH, usually well above 10. The titration from the phenolphthalein to the Methyl orange end point (pH 4.5) represents one-half of the carbonate alkalinity.

**Carbonate-bicarbonate.** Samples containing carbonate and bicarbonate alkalinity have a pH > 8.3 and usually less than 11. The titration to the phenolphthalein end point represents one0half of the carbonate.

**Bicarbonate only.** Samples containing only bicarbonate alkalinity have a pH of 8.3 or less, usually less. In this case bicarbonate alkalinity is equal to the total alkalinity.

## 12. ACIDITY

The acidity of a water is the capacity of that water to neutralize base.

The acidity is significant because acids contribute to corrosiveness of water.

Acidity and Alkalinity are expressed in mg equivalent H or OH ions consumed per liter of sample. However, for practical reasons (comparison with hardness) these parameters are also expressed in mg $CaCO_3$/l

Example:
Suppose in the titration with N molar HCL, a mL HCL are used for V mL sample. The alkalinity can be calculated with the following formula:

$$= \frac{(a \times N \times 1000)}{V} mmolH^+ / L$$

In case the alkalinity is expressed in mg $CaCO_3$/l; 1 mmol $H^+$ is equivalent to ½ mmol $CO_3^{2-}$ neutralization.

$$CO_3^{2-} + 2H^+ \rightarrow H_2CO_3$$

so, with the molecular weight of $CaCO_3$ being 100 mg/mol, 1 mmol $H^+$ is equivalent to 50 mg CaCO3 and the alkalinity follow from:

$$= \frac{(a \times N \times 50{,}000)}{V} mgCaCO_3 / L$$

## 13. SOLIDS

Dr. Nidal Mahmoud

The environmental engineer is concerned with the measurement of solid matter in a wide variety of liquid and semiliquid materials ranging from potable waters through polluted waters, domestic and industrial wastes, and sludges produced in treatment processes. Strictly speaking, all matter except the water contained in liquid materials is classified as solid matter. The usual definition of solids, however, refers to the matter that remains as residue upon evaporation and drying at 103 to 105 °C.

**Definitions for solids found in wastewater**

**Total solids (TS):** the residue remaining after a wastewater sample has been evaporated and dried at a specific temperature (103 – 105 °C)

**Total volatile solids (TVS):** the solids that can be volatilized and burned off when the TS are ignited (500 ± 50 °C)

**Total fixed solids (TFS):** the residue that remains after TS are ignited (500 ± 50 °C)

**Total suspended solids (TSS):** portion of the TS retained on a filter with a specified pore size, measured after being dried at a specific temperature ((105 °C). the filter used most commonly for the determination of TSS is the Whatman glass fiber filter, which has a nominal size of about 1.58 µm.

**Volatile suspended solids (VSS):** those solids that can be volatilized and burned off when the TSS are ignited (500 ± 50 °C).

**Fixed suspended solids (FSS):** the residue that remains after TSS are ignited (500 ± 50 °C).

**Total dissolved solids (TDS): (TS – TSS):** those solids that pass through the filter, and are then evaporated and dried at specific temperature. It should be noted that what is measured as TDS is comprised of colloidal and dissolved solids. Colloids are typically in the size range from 0.001 to 1 µm.

**Total volatile dissolved solids (VDS):** those solids that can be volatilized and burned off when the TDS are ignited (500 ± 50 °C).

**Fixed dissolved solids (FDS):** the residue that remains after TDS are ignited (500 ± 50 °C).

**Settleable solids:**
Suspended solids, expressed as milliliters per liter, that will settle out of suspension within a specific period of time (one hour under quiescent conditions).

***Note:*** *With the exception of settleable solids, all solids are expressed in mg/l.*

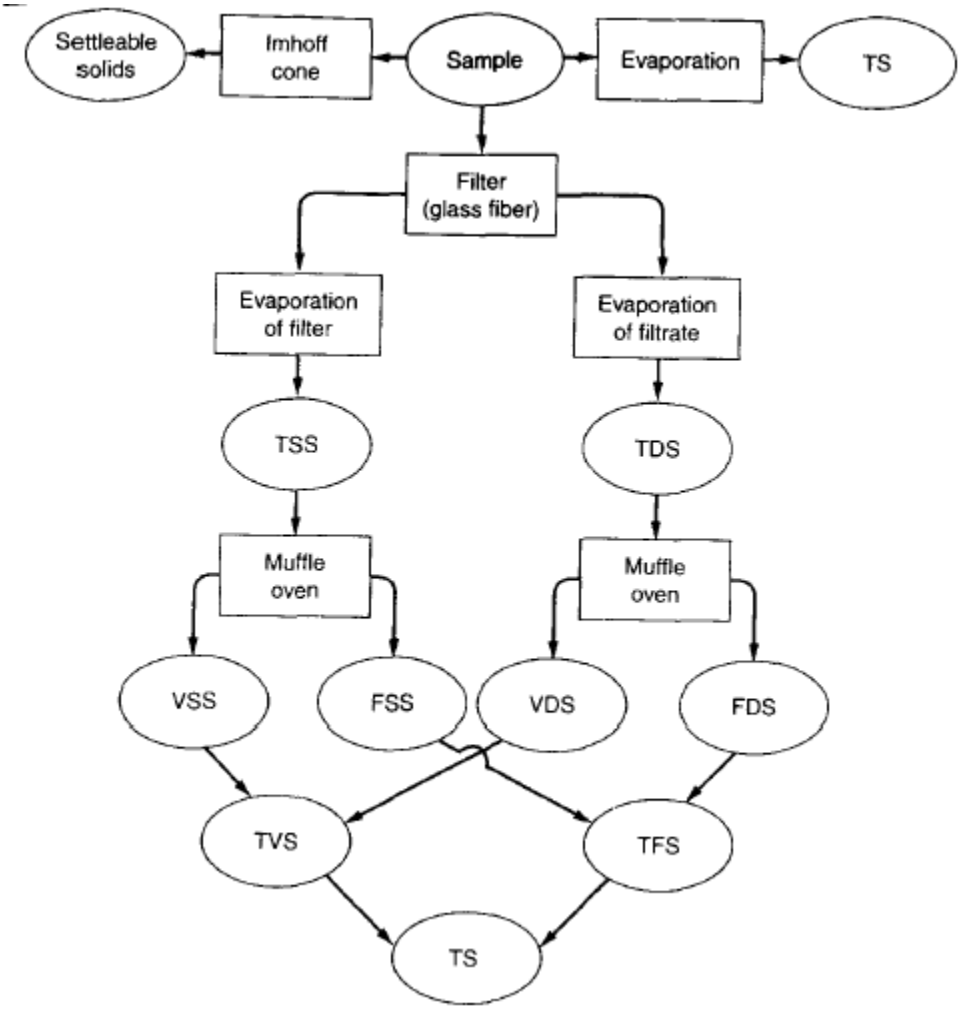


Figure 1. Interrelationships of solids found in water and wastewater.

TS = total solids
TSS = total suspended solids

TDS = total dissolved solids
VSS = volatile suspended solids
FSS = fixed suspended solids
VDS = volatile dissolved solids
FDS = fixed dissolved solids
TVS = total volatile solids
TFS = total fixed solids

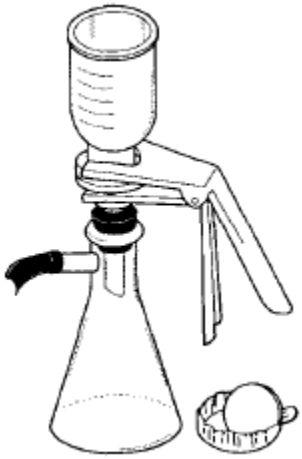

Figure 2. Apparatus used for the determination of total suspended solids. After wastewater sample has been filtered, the preweighted filter paper is placed in a dish for drying before weighing.

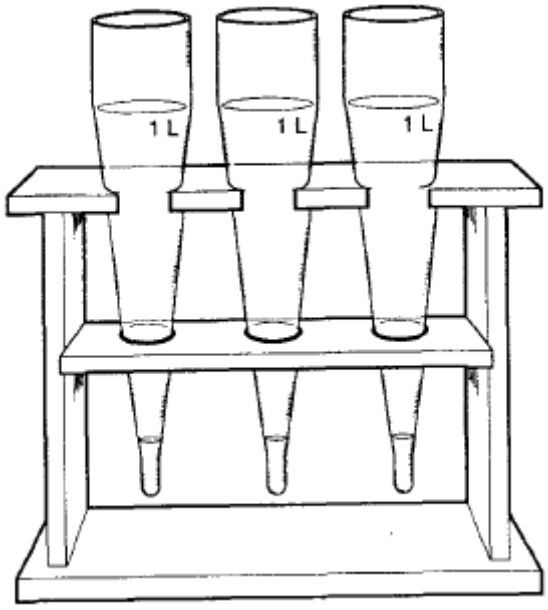


Figure 3. Imhoff cone used to determine settleable solids in wastewater. Solids that accumulate in the bottom of the cone after a specified period of time, and are reported as ml/l.

**Sludge volume index (SVI)**

$SVI_{30}$: the volume of 1 gram TSS after 30 minutes settling in Imhoff cone.

$$SVI = \frac{\text{settled volume of sludge, ml/l}}{\text{TSS, g/l}} = \frac{\text{ml}}{\text{g}}$$

**Environmental significance**

**Dissolved solids.** The amount of dissolved solids present in water is a consideration in its suitability for domestic use. In general, waters with a total solids content of less than 500 mg/l are most desirable for such purposes. Waters with higher solids content often have a laxative and sometimes the reverse effect upon people whose bodies are not adjusted to them.

**Settleable solids.** The settleable solids test is used to determine the efficiency of sedimentation tanks and to determine the need for and design of sedimentation tanks in water treatment facilities.

The **Suspended- and volatile- suspended solids** determinations are used to evaluate the strength of domestic and industrial wastes.

The **Total- and volatile –solids** tests are normally applied to sludges. They are indispensable in the design and operation of sludge-digestion, vacuum filter, thickener and incineration units.