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INSTRUCTIONS

FOR THE RESEARCH ON WASTEWATER TREATMENT FOR DEVELOPING COUNTRIES

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WAGENINGEN SEPTEMBER,1985

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INSTRUCTIONS
FOR THE RESEARCH ON WASTEWATER TREATMENT
FOR DEVELOPING COUNTRIES

This report is one of the activities of the Inter-universitary project VH-17 between Wageningen Agricultural University, the Netherlands and HochiMinh City Polytechnic University, Vietnam.

J.C.L.van BUUREN
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INTRODUCTIONS FOR THE RESEARCH ON WASTEWATER TREATMENT FOR DEVELOPING COUNTRIES.

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INTRODUCTION

This report gives instructions to students and scientists in developing countries how to plan and carry out research in the field of waste water purification.

This research can be undertaken with the purpose to advance certain waste water technologies.More often,however, it will be research to investigate which of the methods known from literature are suitable for particular types of waste water.
How these methods can be adapted to local needs and circumstances?.
Such investigations can be carried out on a laboratory or on a semi-technical scale.

Our instructions are preced by an overview of purification methods which are widely considered as appropriate for developing countries, and by some important methods of waste-water analysis.
Also a methodological introduction in the formulation of research questions is included.

The following water treatment technologies are discussed here:

- Anaerobic treatment of domestic and industrial waste water and of manure.Special emphasis is laid on the Upflow Anaerobic Sludge Blanket process(UASB),
- Facultative and aerobic stabilisation ponds,
- Biofiltration (also called trickling filters)
- Intermittent slow sand filtration.

Of course this list is not exhaustive as appropriate technologies are concerned.

This report was produced within the framework of the interuniversitary project Biogas Generation and Waste Water Treatment in Vietnam.
In this project the Department of Water Pollution Control of the Agricultural University Wageningen(AUW) and the Department of Environmental Engineering of the Polytechnic University of Ho Chi Minh City cooperate during a period of three years (1985 - 1987).
We expect that a guide for starting research in the field of waste water treatment will meet a substantial need of scientists in charge of environmental improvements in developing countries.
This is a first draft.Our purpose is to give it a more definitive form on the basis of the experiences of the users in Vietnam.

1.WASTEWATER TREATMENT : AN OVERVIEW

Wastewater collected from cities,towns and factories must ultimately be returned to receiving waters or to the land.The complex question of which contaminants in wastewater must be removed to protect the environment-and to what extent-must be answered specifically for each case.This requires analysis of local conditions and needs,together with the application of scientific knowledge,engineering judgment based on past experience, and consideration of federal and state requirements and regulations.

1.1.Anaerobic treatment processes.

Anaerobic treatment is the use of biological processes in the absence of oxygen for the stabilization of organic materials by conversion to methane and inorganic end products including carbon dioxide and ammonia.
In nature,anaerobic digestion is part of the carbon cycle as it plays a role in the mineralization of organic material.Anaerobic decomposition of organic materials occurs at all places where organic matter accumulate and the supply of oxygen is insufficient for aerobic degradation,e.g. in mashes and lake sediments.

Early years with anaerobic treatment:

Early anaerobic treatment systems which combined sedimentation and digestion in a single unit,(Figure 1.1,1.2,1.3).

Septic tank :The effluent from septic tanks often was black and offensive, and still contained undigestable material which clogged contact beds often used for further subsequent treatment.

Travis tank :In the Travis tanks the suspended material was separated from the wastewater and allowed to pass into a separate "hydrolyzing chaber".

Imhoff tank :In the Imhoff tanks,the wastewater was not allowed to flow through the "hydrolyzing"chamber.The sludge was allowed to stay in this chamber from a few weeks to several months,and when in proper condition it was inoffensive and could be disposed without nuisance.

More recent process developments:

Figure 1.4,1.5 show the Conventional and high-rate separate digestion

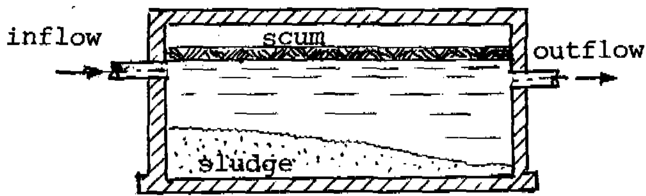


Fig.1.1.Septic tank

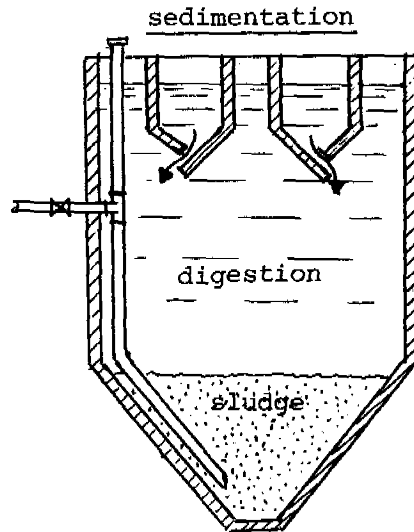


Fig.1.3.Imhoff tank.

Wastewater flows through sedimentation chambers only

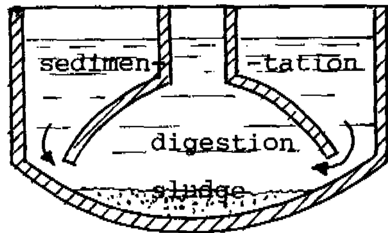


Fig.1.2.Travis tank.

Wastewater flows through all three chambers.

systems,commonly used for sewage sludge digestion.

In the conventional-rate digestion process,the contents of the digester are unheated and unmixed.This resulted in separation of solids from the liquid forming a thickened sludge at the bottom of the tank and a floating scum layer at the top.

In the high-rate digestion process,the contents of the reactor are heated and completely mixed.This resulted not only removed the scum layer,but enhanced the rate of digestion by bringing bacteria and wastes more closely together.

Figure 1.6,1.7 show the suspended-growth digesters designed to maintain high bacterial populations,allowing digestion at shortened hydraulic detention times:anaerobic contact process and upflow anaerobic sludge blanket (UASB)reactor.The UASB reactor is one of the reactor types with a high loading capacity.It's process is charaterized by a reactor containing no packing or any other type of biomass support material.No separate settler with sludge return pumps is required.

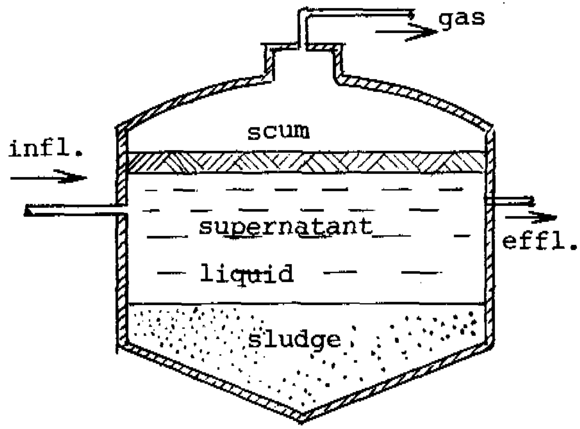


Fig.1.4.Conventional digester

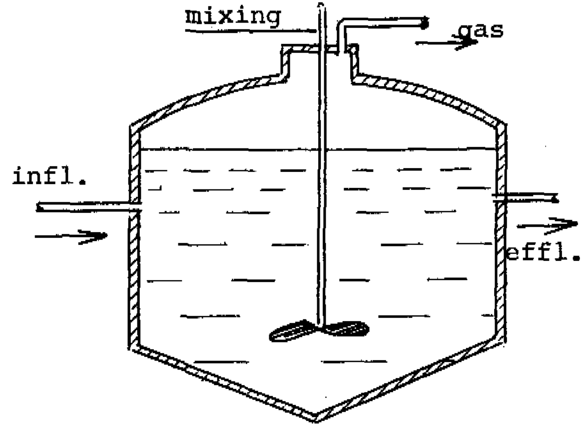


Fig.1.5.High-rate digester

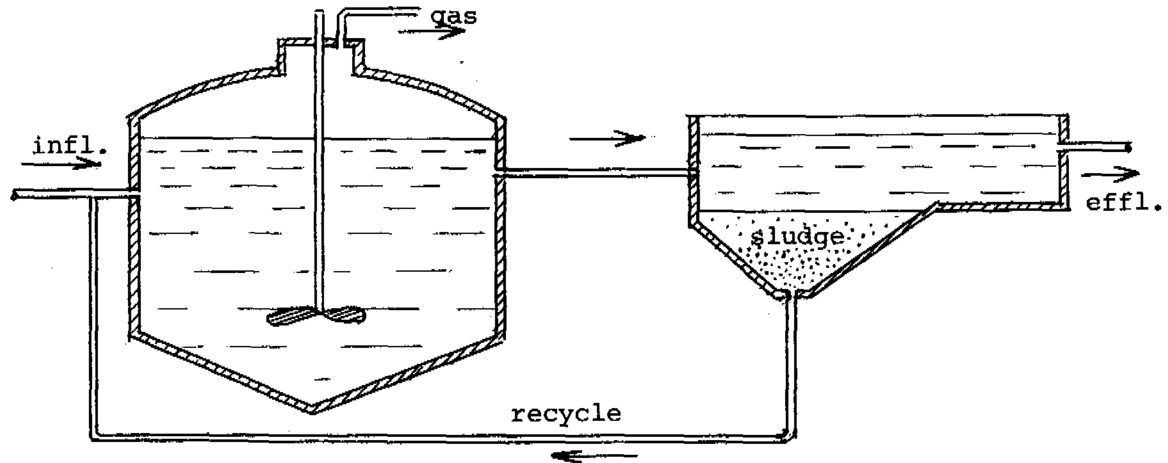


Fig.1.6. Anaerobic contact process

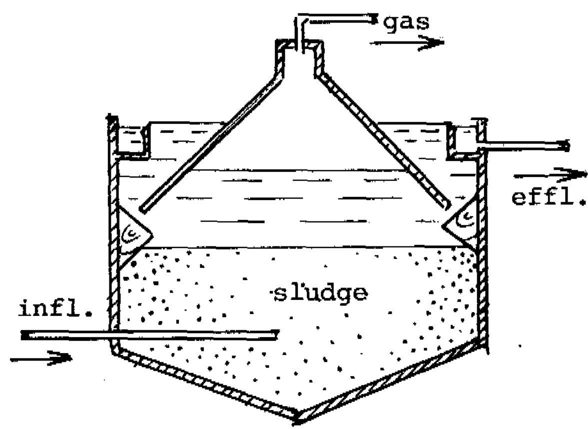


Fig.1.7.Upflow Anaerobic Sludge Blanket

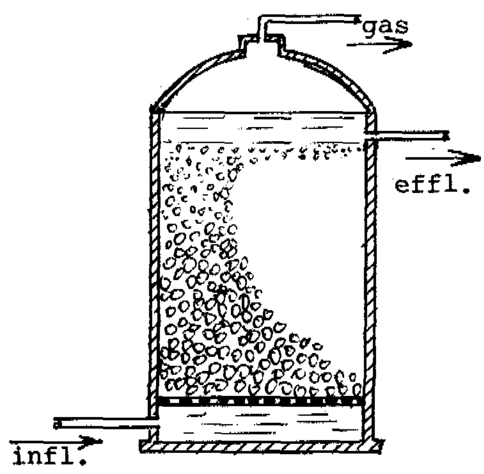


Fig.1.8.Anaerobic Filter

In the UASB reactor (F.1.7) the biomass retention is obtained via settle - ment of the sludge with the aid of an internal settling compartement. With a long solid retention time (SRT),high hydraulic rates can be obtained. As a result,anaerobic treatment of more dilute wastewater becomes attractive. The UASB-concept is so far the most applicated full scale high-rate anaerobic treatment process.The UASB -concept is feasible varety of wastewater, like sugar beet,potato processing,corn and potato starch and distillery wastes.

1.2.Process microbiology

The biological conversion of complex organic matter to methane and carbon dioxide depends on the combined activity of a miscelanneous microbial population,consisting of diverse genera of obligate and facultative anaerobic bacteria.

The responsible microbial population exists of at least 4 trophic groups of bateria,each with a distinct carbon catabolizing funtion:Hydrolytic bacteria,Acidogenic bacteria,Acetogenic bacteria,and Methanogenic bacteria.

Hydrolytic bacteria (group 1) ferment organic polymers,e.g.proteins,polysaccharides and lipid materals into compounds with a fewer mol-weight, uch as volatile acids,organic acids,alcohols,hydrogen,carbon dioxide,ammo -nia nitrogen and sulphide.

Acidogenic bacteria (group 2) degrade propionate and longer chain fatty acids,alcohols,aromatics and other fermentation products.They produce acetate,hydrogen and carbon dioxide.Because of their metabolic activity, the bacteria form an intermediate group linking the fermentative and methanogenic stage.

The acetogenic bacteria (group 3)show a mixotrophic metabolism and catabolize both hydrogen/carbon dioxide and muticarbon compounds.They can produce acetate and longer chain volatile acids from hydrogen/carbon dioxide mixtures,but are successfully outcompeted for hydrogen by the me - thanogenic bacteria in the gastro-intestinalother environments where methanogenesis is fount.

The methanogenic bacteria (group 4)produce methane from acetate and one-carbon compounds,e.g.hydrogen/carbon mitures,methanol,formate and methylamine.The methanogens are the key-organisms in the anaerobic digestion process providing thermodynamically favourable conditions for the pre - ceeding non-methanogenic stage.
The main methanogenic and acetogenic reactions are given in Table 1.1.

TABLE 1.1. Methanogenic and Acetogenic reactions.

SUBSTRATE	REACTION
METHANOGENIC	
Acetate	$CH_3COOH \longrightarrow CH_4 + CO_2$
hydrogen	$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O$
methanol	$4CH_4OH \longrightarrow 3CH_4 + CO_2 + 2H_2O$
ACETOGENIC	
ethanol	$CH_3CH_2OH + H_2O \longrightarrow CH_3COOH + 2H_2$
propionate	$CH_3CH_2COOH + 2H_2O \longrightarrow CH_3COOH + 3H_2 + CO_2$
butyrate	$CH_3CH_2CH_2COOH + 2H_2O \longrightarrow 2CH_3COOH + 2H_2$
$CO_2 + H_2$	$2CO_2 + 4H_2 \longrightarrow CH_3COOH + 2H_2O$

1.3.The aerobic purification of waste water

Many methods for the treatment of waste water are based on the activity of aerobic microorganisms.Here,we give some information about three of these methods:stabilisation ponds,biofiltration and intermittent slow sand filtration.These methods satisfy criteria of suitability for tropical climates,low investment and maimtenance cost amd low energy input.These methods can be used either as main treatment method for COD and ammonia removal or as posttretament step after an anaerobic pretreatment.

1.3.1.Waste stabilisation ponds

Simple waste water ponds ,if they are well designed,can be a very effective treatment method.It depends on the relationship between the BOD-load of the influent flow and the surface area of the ponds whether the ponds will be anaerobic,facultatively aerobic (this is: partially aerobic (at the surface) and partially anaerobic (at the bottom)) or completely aerobic,

The basic purification mechanism in the facultative and aerobic pond is given in figure 1.9.

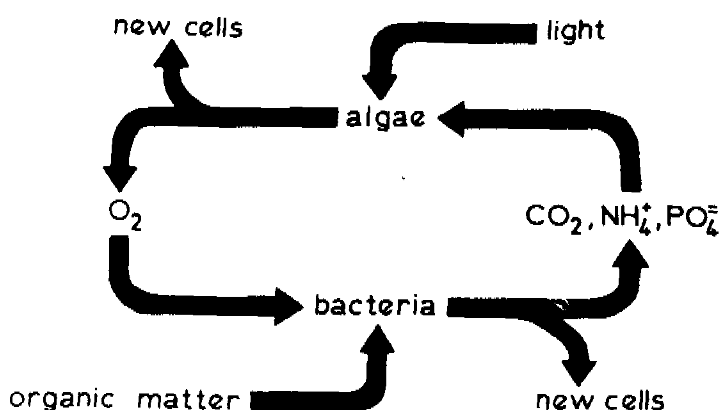


Fig.1.9.Symbiosis of algae and bacteria
Source: From Mara (1977).

Algae produce the oxygen (photosynthetic activity) to be used by the bacteria in order to convert the organic matter from the influent.

Design considerations

If waste water stabilisation ponds are applied as the only purification method,the plant lay-out will be as follows:

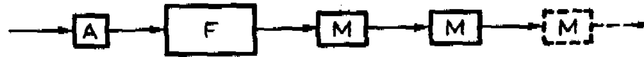


Fig.1.10.Pond layouts.A,anaerobic pond;F,facutative pond; M,maturation pond.

The first pond/s will be anaerobic because of the relatively high organic load.The depth of this pond will be approximately 4 m .At a residence time - of about 5 days the BOD removal will be about 70%.
The second pond will have a facultatively aerobic character.Its main function is BOD removal.The third pond in this series is called maturation pond.
It will be completely aerobic and serve as a posttreatment pond in which the remaoval of (pathogenic) microorganisms and nitrification are the main processes.Facultative and maturation ponds usually have a depth of 1 - 1.5 m.
Too shallow ponds give too much plant growth and need a lot of surface area.
Too deep ponds tend to become anaerobic as light has to penetrate until the bottom.There are different approaches to the design of aerobic ponds.
As a first approximation one may use the first order kinetics of the BOD removal.

This figure schematically shows the surface of the pond and the influent and effluent flows.

L_i = BOD-concentration influent (mg/l)
L_e = ,, ,, effluent (mg/l)
Q = waste water flow (m^3/day)
V = pond volume = A.d (m^3)

A = surface area of the pond(m^2)
d = pond depth (m)
K_1= first order rate constant(day^{-1})
t = residence time (= V/Q = A.d/Q(day)

1.3.

The mass balance equation over the pond is:

$$Q.L_i = Q.L_e + K_1.V.L_e \qquad \text{(gr/day)}$$

In words: $\text{BOD-load}_{\text{influent}} = \text{BOD-load}_{\text{effluent}}$ plus BOD removed

From this equation the relationship between influent and effluent BOD-concentration can be derived.

$$\frac{L_e}{L_i} = \frac{1}{1 + K_1.t} = \frac{1}{1 + K_1.V/Q}.$$

K_1 is dependent on temperature: $K_1 = 0.3\ (1{,}05)^{T-20^o}\ (\text{day}^{-1})$.
(T = temperature in degrees Celsius)
At 28^oC : $K_1 = 0.3(1.05)^{28-20} = 0.44\ \text{day}^{-1}$.
For design purposes the temperature of the coldest month should be taken.

A series of two or more ponds with the same surface area as one big pond has a better treatment efficiency than the one big pond because the BOD-level in the ponds preceding the last pond will be higher.
One can also say that a series of ponds better approximates a plug flow type of reactor:and this implies a far higher removal if first order kinetics may be assumed.
The overall treatment efficiency for n ponds in series can be calculated from

$$\frac{L_e}{L_i} = \frac{1}{(1 + K_1.V/Q)^n}$$

n = number of small ponds in series.
V = volume of one small pond. (m^3).

<u>Example</u>

Calculate the pond surface of a facultative pond system loaded with an influent $L_i = 400\ gr/m^3$.$Q = 1{,}000\ m^3/day$; $K_1 = 0.4$ in the coldest month; depth= 1m.
The effluent should have a BOD of $20\ gr/m^3$.
If one pond will be used it can be calculated that:
$K_1.V/Q = 19$. This means $t = V/Q = 19/0.4 = 47.5$ days.
$V= 47.5 \times 1{,}000\ m^3 = 47{,}500\ m^3$. A = 4.75 ha.

If two ponds are plannned: $(1 + K_1.V/Q)^2 * 0.05 = 1$.
So: $K_1.V/Q = 3.47$. t= 8.7 daysper pond.

For two ponds the total residence time is: 17.4 days.The surface area will be 1.74 ha.

Conclusion:the advantage of constructing two ponds in series is obvious.

1.3.2.Biofiltration

The biofilter,also known as trickling filter,consists of a circular or rectangular bed of coarse filtermedium (e.g. volcanic stones) on which the settled waste water is distributed.The filter depth usually is about 2 meters.The filter medium has a grading of 30 - 100 mm.
In order to avoid too rapid clogging of the filter bed the waste water has to be pretreated by screening,grit removal and sedimentation, or by screening and anaerobic treatment in an UASB-reactor.

After its distribution over the filter surface the waste water trickles down through the filter medium.There are always enough void spaces in the filter medium to supply oxygen to the water.
On the surfaces of the filter medium a microbial film develops and the bacteria which constitute most of the film oxidize the waste water as it flows past.As the waste material is oxidized the film grows.
Some of the microbial film is washed away by the hydraulic action of the passing waste water.The effluent usually contains a considerable concentration of suspended matter which can be removed by sedimentation.
The usual lay-out of a biofiltration unit is as follows:

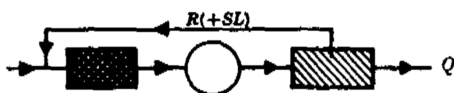
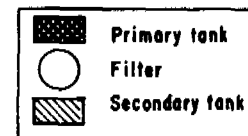


Fig.1.11. Lay-out of trickling filter and sedimentation tanks.



A part of the effluent is recirculated to the filter to avoid filter blockage by excessive growth of the microbial film.The recirculation also serves a better distribution of the influent over the bed surface and the bed depth.A disadvantage of recirculation,particularly in developing countries,is the extra energy input and the need of skilled labour.

and a flow equalisation.

1.3.

Biofiltration without recirculation (so called single pass filtration) is possible but the organic loading should be limited in that case. The following table presents some data on typical organic and hydraulic loadings for low-rate single pass and high-rate recirculated biofilters.

TABLE 1.2. Data on typical organic and hydraulic loadings of trickling filter

	Low-rate single pass filter	High-rate filter with recirculation
Organic load (BOD-load)	0.08 - 0.4 $kg/m^3.day$	0.4 - 50 $kg/m^3.day$
Hydraulic load	1 - 4 $m^3/m^2.day$	8 - 40 $m^3/m^2.day$
Typical BOD-removal after sedimentation	90 %	65%

Example

Calculate the diameter of a circular single pass trickling filter for a flow of sewage of 100 m^3/day and with a BOD concentration of 300 mg/l(gr/m^3).

The organic load will be 100x 300 gr/day = 30 kg/day.

The filter will be designed for a typical load of 0.2 $kg/m^3.day$

The filter volume should be: 30/0.2 = 150 m^3.

If the depth is 1.8 m ,the surface area will be 150/1.8 = 83 m^2 and the diameter 10.3 m.The hydraulic load is 100/ 83 m/day = 1.20 m/day. The hydraulic load should not be too low in order to avoid blockage. Stronger wastewater could easily lead to rapid deterioration of the biofilter.

If the BOD-load is relatively low and the bed not too shallow nitrification may result.

1.3.3. Intermittent slow sand filtration

Intermittent slow sand filtration was developed from land treatment of waste water in the last century.By using graded sand instead of soil and supplying the waste water intermittentlythe filtration capacity could be improved much.The intermittent mode of filtration allows of air entering the filter medium during the dry periods.
As in land treatment the removal efficiencies for several waste water parameters are high.Sand filters consist of rectangular beds filled with graded sand (effective diameter 0.16 - 0.35 mm ,uniformity : 2) to a depth of approx. 0.9 m.The waste water which has undergone some sort of pretreatment is supplied to the beds until they are covered to a depth of 50 - 100 mm.The hydraulic load of the beds is mainly determined by the degree of pretreatment and the suspended solids and BOD-concentration of the influent.
The filter regime ,this is the cycle of wet and dry periods,may vary with load but normal practice for secondary waste water is a dosing of once or twice a day.

The filter action is determined by the following processes:sieving of relatively big particles at the filter surface,interception of paticles in the filter pores,BOD- and ammonia removal by the aerobic filter flora,mineralisation of suspended matter ,also by the filter flora.
In the removal of pathogenic bacteria the protozoa play an important role.
At relatively high loads of organic matter a filter may become clogged.
Filter maintenance consists of raking of the surface or taking away the top layer of the filter sand for cleaning.This must be done as soon as the capacity has dropped to about 40% of the original capacity.

Design considerations

In the design of an intermittent slow sand filter (ISF) the load of suspended solids (SS) usually is the most critical parameter.
That is :in the treatment of primary of secondary effluent the clogging (also called plugging) of the filter,which decreases the capacity of the filter,is mainly caused by the supply of SS and only to a lesser degree by the bacterial growth on soluble substances(BOD and NH_4^+).
The quantity of wastewater which can be applied until the moment of clogging can be derived from the loading with suspended solids(gr/m^2.day)

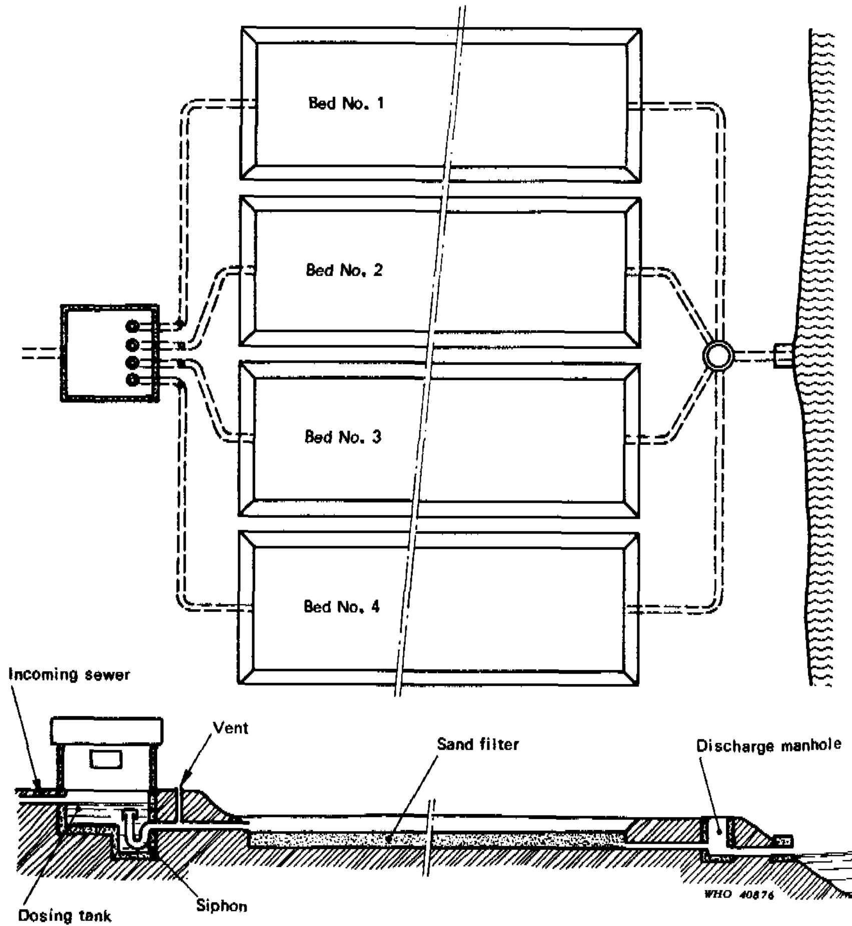


Figure 1.12

Lay-out for intermittent sand filtration plant.

(From:D.A.Okun,G.Ponghis,Community Wastewater Collection and Disposal,WHO Geneva,1975).

The following relationship between filter running time and SS loading was found by Cowan and Middlebrooks(1980).

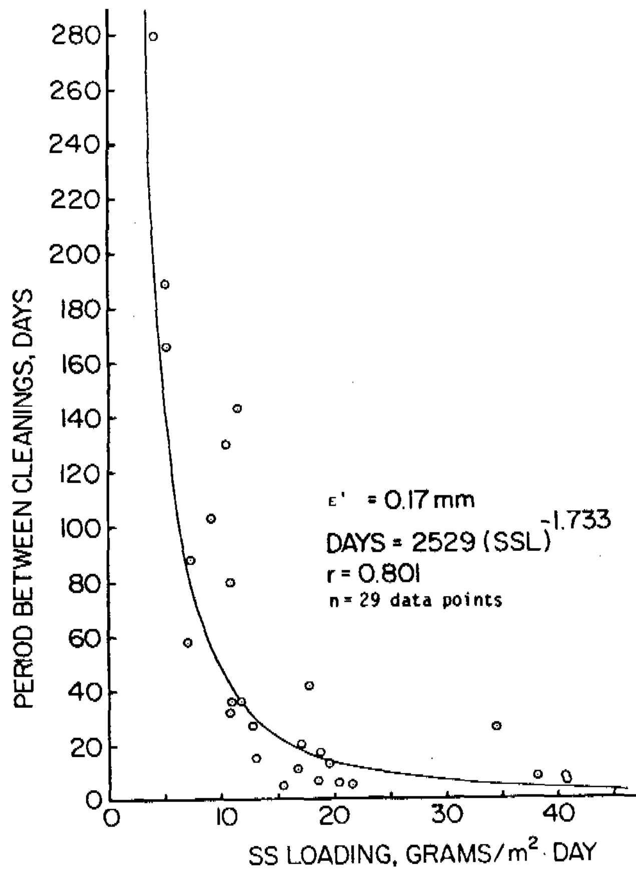


Fig.1.13.Period of filter operation as a function of SSL for filters containing 0,17 mm effective size sand.

This graph can be fitted into the equation:

$$\text{Days to plugging} = 2529.\ (SSL)^{-1.733}.$$

The moment of clogging is defined as follows.The influent is put onto the filter once per day.If the infiltration becomes so slow that the water cannot infiltrate completely before the next batch is put onto the filter after(24 hrs)the filter is said to be clogged.

For other sand sizes (0.40 and 0.68 mm) the following equations were found:

$$\text{Days to plugging} = 8859.(SSL)^{-1.625} \quad (\text{Eff. diameter} = 0.40\ mm)$$

$$\text{Days to plugging} = 12{,}350.(SSL)^{-1.445} \quad (\text{Eff. diameter} = 0.68\ mm).$$

ISF can be used as a secondary treatment step for the removal of organic matter after the primary removal of settleable solids in a sedimentation tank or a septic tank.Also the Anaerobic Upflow Reactor (UASB) can be used as a primary treatment preceding the sand filter. ISF can also be used as a tertiary treatment process after preliminary treatment in for instance an activated sludge process or in stabilisation ponds.

Although the hydraulic capacities of slow sand filters are usually low (5 - 100 cm/day) and the filter surface areas correspondingly large, the strong point of filters is their good effluent quality,particularly where fine sands are used (Eff. diameter approx. 0.2 mm)
The effluent can be fully nitrified with a SS of $\pm$ 5 mg/l ,a BOD of $\pm$ 10 mg/l and coliforms Most Probable Number of smaller than 100/100 ml. (100/100 ml is the coliform standard for unrestricted irrigation). Effluents of this high quality are required where reuse is considered. E.g. reuse for aquaculture,aquifer recharge and irrigation.

Example

Calculate the surface of filterbeds which will have to treat anaerobicall pretreated domestic wastewater.Waste water flow: 100 m^3/day. SS -concentration: 60 mg/l.The BOD = 100 mg/l.Coliform MPN = 10^6/100ml. Use the figure above.Chose as the desired period of operation: 60 days. The acceptable daily SS loading is 10 gr/m^2.day.
This means an hydraulic loading of: 10/0.06 l/m^2.day = 167 l/m^2.day.
This means a surface area of 100/0.167 m^2 = 600 m^2.
As approximately ¼ of the filter area must be kep unloaded for cleaning purposes the total filter surface area should be 4/3 x 600 = 800 m^2.

The removal of suspended solids by gravel filtration

As a simple pretreatment of the influent for an intermittent sand filter the pebble bed clarifier or the horizontal rock filter may be used.Duncan Mara (Sewage Treatment in Hot Climates,1976) describes these processes as follows.

The pebble bed clarifier

This is an upward flow clarifier in which the effluent passes through a 150 mm layer of pea gravel (6 - 9 mm grading) which is supported by steel mesh.(See figure below: 1.14)
At a hydraulic loading of 20 - 25 m^3/m^2.day SS removal is about 50 per cent. The solids which accumulate in the bed are flushed out by a jet of water (or effluent) when the water level has been reduced to below the bed.
The experience with this kind of filter at AUW showed the same SS removal from UASB effluent but the hydraulic load was much lower: 4 m^3/m^2.day.

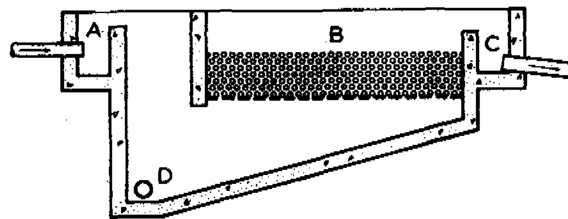


Fig.1.14.Banks filter.A,inlet weir;B,pebble bed on steel mesh support;C,efflent weir;D,sludge drain.

The horizontal rock filter

An extension of the pebble bed clarifier is the horizontal rock filter (35 - 50 mm grading) proposed for removing algae from stabilisation pond effluent as it leaves the pond (See figure1.15)
Approximately 24 h retention time in the filter should be provided;assuming a voids ratio of 45% ,a filter volume of 0.18 m^3/capita is required for a waste flow of 80 l:cap.day.The rock filter therefore is best suited to small works.

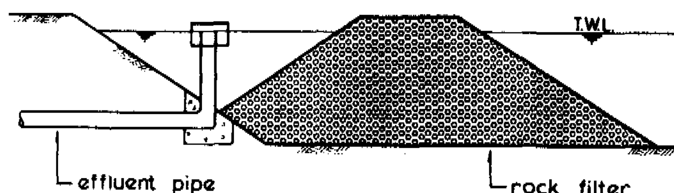
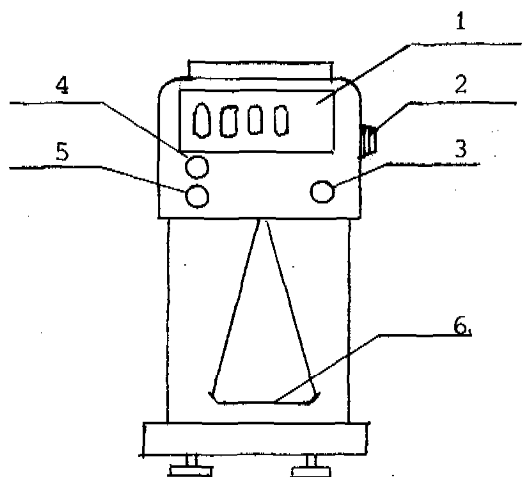


Fig.1.15. Horizontal rock filter for algae removal from waste stabilization pond effluent

2. MAIN EQUIPMENTS AND APPARATUS FOR ANALYSIS AND DETERMINATION

Figure 2.1-2.25 show the equipments and apparatus which are used for determinations of the main parameters of wastewaters and wastewatertreatment.
Figure 2.1 shows the analytical balance. It is used for weighing with a very high precision. With the analytical balance you can weigh a reagent or other substance up to 0.1 mg precise.
In Fig. 2.1 : - 1 display (weight) 2 - on/off , tare knob,3-5 are used after taration to switch on the right weight on the display. 6-balance-pan. Fig. 2.2 shows the display of the analytical balance.
Figure 2.3 shows the technical balance. It is usedfor weighing relative large amounts of substances when a high precision is not needed.
Fig. 2.3 : 1-pan 2- display (detail Fig 2.4) 3-5- switchesfor turning the weight after taration 4-on/off, tare switch.
Fig.2.5 shows the electronic macrobalance. This balance is used for weighing large amounts (up to 15 kg) like substrate vessels., when a low precision is no objection. This balance weighs up to 1 g precise.
Fig. 2.6 shows the pH-meter + pH-electrode.It is used to determine the pH of all kinds of samples, or direct measurements in reactors.
1- calibtation switch pH 7, 2- switch for different pH-ranges (6-10, 0-8 6-14),3- swich for the temperature of the sample, 4- calibrationswitch pH 4.00.
Fig. 2.7 Funnel .
Afunnel is used for:a) to pour fluids through narrow openings
b) to hold a filterpaper for filtration
Fig.2.8 Time-clock. A time clock is used when electric apparatuslike pumps or stirrers must be run intermittently.E.G. stirring every half an hour for 10 seconds. 1- pause switch 2- puls switch
Fig. 2.9 shows a Heidolph srring engine. This stirring engine is used for stirring batch reactors substratevessels, UASB-reactors, etc.
It is possible to turn the engine into a pump by a small adaptation.
In Fig. 2.10 an exsiccator is shown. This apparatus is used for cooling samples,which are heated in a stove, to roomtemperature. To prevent uptake of water by the sample (hygroscopic effect) a drying material is placed in the exsiccator. After cooling a sample in this way the weight of a sample can be determined accurately.

In Fig. 2.11 an oven is shown. This apparatus is used for heating samples during the determination of the ash-free weight of a sample (sludge, or other organic matter).
Fig. 2.11: 1- oven-door,2-opening handle,3-switch for controlling temperature,4- on/off switch,5- lamp indicator heating 6-temperature display.
In Fig.2.12 a condensing cooler is shown. This cooler is used when a sample is heated for a certain time. The volatile components which fly from the sample condensate in the cooler and flow back in the liquid phase
In this way most components of the sample will stay in the sample.
In Fig 2.13 a titration equipment is shown. This automatic titration apparatus is used for determinations with a lot of samples to titrate e.g. COD determinations.
Fig. 2.13: 1-titration pump,2-display titration volume,3-speed addition controll,4-on/off,manual/automatic controll,5-reset display switch, 6piston,7-tube titration volume,8 variable two way piece,9-tube,10-stock titration fluid,11-glasspipet point,12 flask magnetic stirring engine.
In Fig. 2.14 a dispensor is shown. It is used for rapid addition of reagents of a ceertain volume.
Fig.2.15 Conical flask. This flask is used for many purposes, like titrations etc.Fig. 2.16 Siphon is used for additions of demineralised water to samples, for washing etc.
Fig. 2.17 Pipeting balloon. This balloon is used when toxic reagentsor other dangerous chemicals have to be pipetted.
Fig.2.18 Pipet. A pipet is used when an exact volume of a sample is used for a determi-ation.
Fig.2.19 Buret. Aburet is used for simple titration methods and to add reagents or titration fluids in an accurate way.
Fig. 2.20 Meauring flask. This flask is used to prepare solutions in an accurate way., e.g. very diluted solutions.
Fig. 2.21 Measuring cylinder. This cylinder is used for measuring certain amounts of a sample (larger then 50 ml) or reagents.
Fig. 2.22 Syringe. A syringe is used for sampling or adding very small amounts of a sample or reagents, e.g. for gaschromatography.
Fig 2.23 Membranefilter. This object is used when samples must be free of solids or bacteria
Fig. 2.24 Small bottle for samples analysed by gaschromatography
Fig. 2.25 Small bottle for SO_4^{2-} samples with high performance liquid chromatography (HPLC).



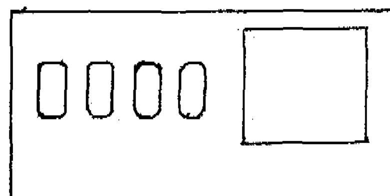
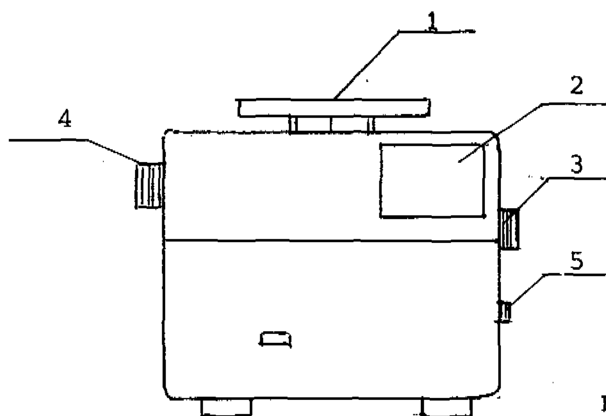


Fig.2.2.Display of analytical balance

Fig.2.1.Analytical balance



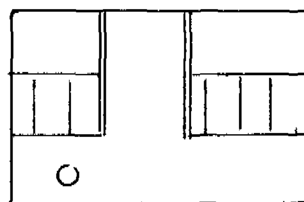


Fig.2.4.Display of technical balance

Fig.2.3.Technical balance

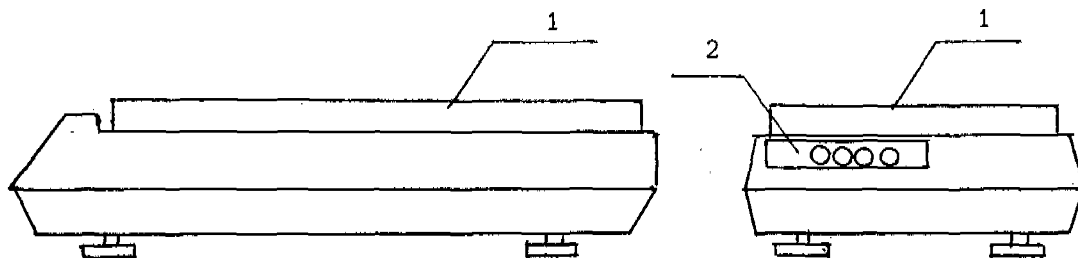


Fig.2.5.Electronic macro balance

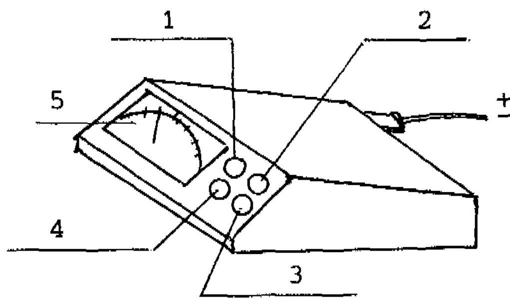


Fig.2.6.pH-meter+pH-electrode

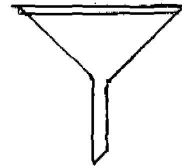


Fig.2.7.Funnel

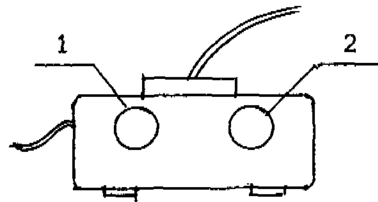


Fig.2.8.Time Oclock

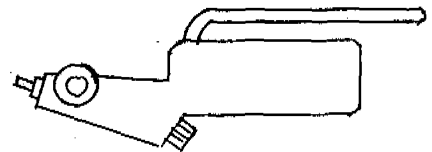


Fig.2.9.Heidolph stirring engine

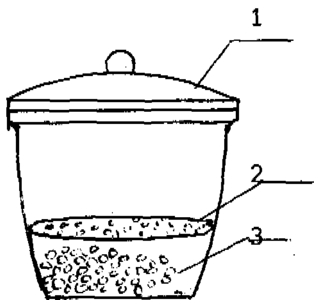


Fig.2.10.Exsiccator

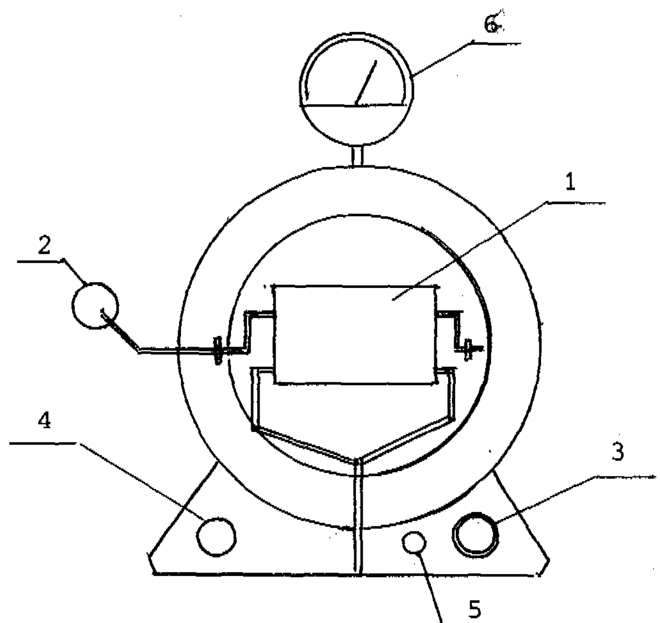


Fig.2.11.Oven 0-1000°C

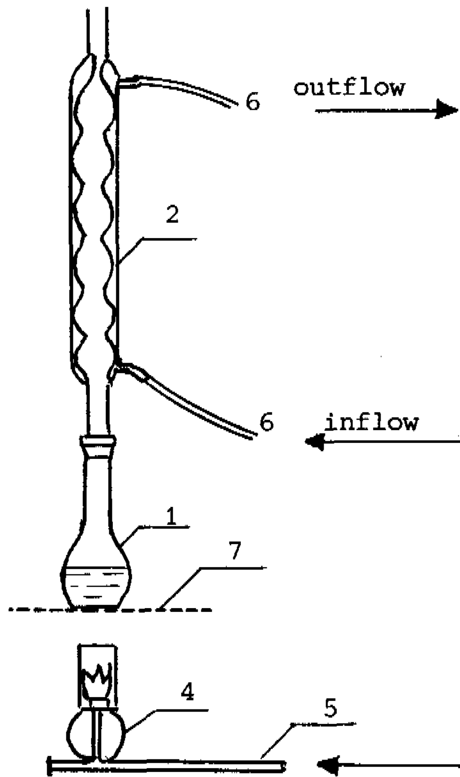


Fig.2.12.Condensing cooler

1.COD-flask,

2.cooling tube (condenser),

3.flame spreading device,

4.gas burner,

5.gas supply,

6.tap-water flow.

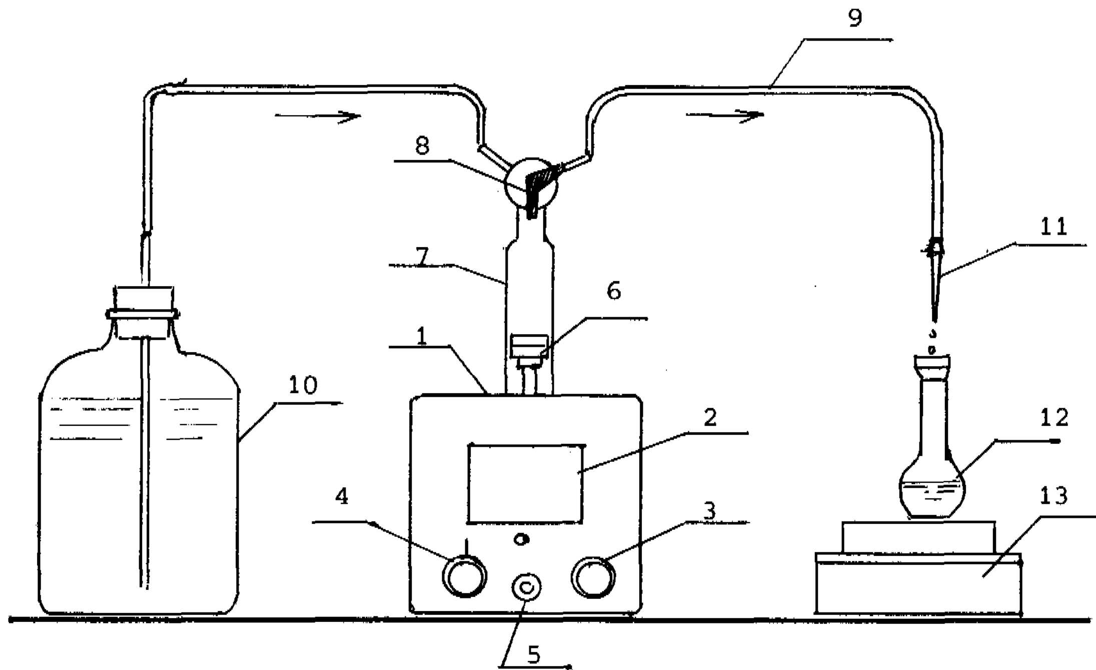


Fig.2.13.Titration Equipment

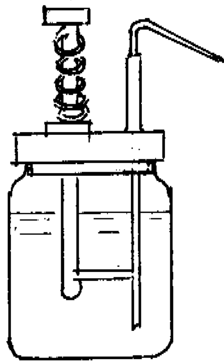


Fig.2.14.Dispensor

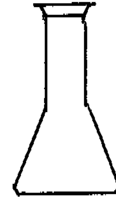


Fig.2.15.Conical flask

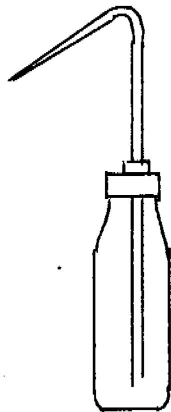


Fig.2.16.Siphon

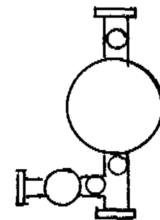


Fig.2.17.Pipeting balloon

Fig.2.18.Pipet

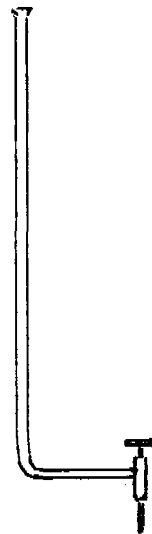


Fig.2.19.Buret

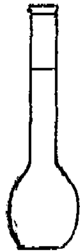


Fig.2.20.Measuring flask

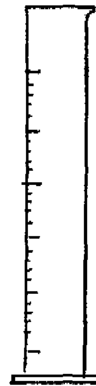


Fig.2.21.Measuring cylinder

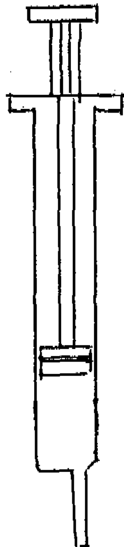


Fig.2.22.Syringe

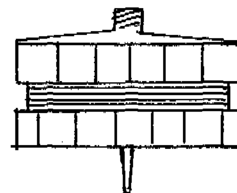


Fig.2.23.Membrane filter

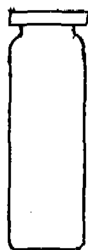


Fig.2.24.Small bottle for VFA sample (by gas chromatograph)

Fig.2.25.Small bottle for SO_4^{-2} sample (by Computing integrator)

3.DETERMINATIONS OF MAIN PAPAMETERS

3.1.Oxygen demand (chemical),COD.

The chemical oxygen demand (COD)determination is a measure of the oxygen equivalent of that portion of thr organic matter in a sample that is susceptible to oxidation by a trong chemical oxidant.It is an important,rayidly measured parameter for stream and industrial waste studies and control of wastewater treatment plants.

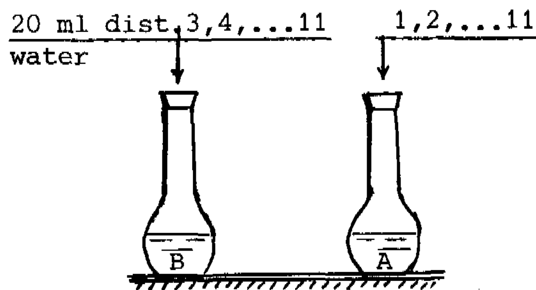
Apparatus

Reflux apparatus (Fig.2.12)

Reagents :Standard potassium dicromate solution,0,25N;Sulfuric acid reagent conc H_2SO_4 containing silver sulfate,Ag_2SO_4;Standard ferrous ammonium sulfate titrant,0,1N;Ferroin indicator solution;Mercuric sulfate,$HgSO_4$crystal

Procedure

Fig.3.1.shows the COD-flask : A-for sample,B-for blank.



.Fig.1.3. COD- flask

For A :

1.place 2 ml of sample,

2.dilute 18 ml distilled water,

3.place some Hg_2SO_4,

4.several boiling chips,

5.2 ml H_2SO_4 18 mol.Add the H_2SO_4 very slowly,with mixing to dissolve the H_2SO_4,

6.add 10 ml 0,25N $K_2Cr_2O_7$,

7.add 30 ml $(Ag_2SO_4+H_2SO_4)$.

8.attach the flask to the condenser and start the cooling water (F.2.12).

Mix the reflux mixture thoroughly before heat is applied;if this is not done,local heating occurs in the bottom of the flask and the mixture may be blown out of the condenser,

Reflux the mixture for two hours,

9.Cool and then wash down the condenser with distilled water,

10.Dilute the mixture with distilled water to about 150 ml,

11.Cool to the room temperature.

Reflux in the same manner a blank (Fig.3.1B) consisting of 20 ml distilled water, equal in volume to that of the sample, together with the reagents. Then titrate the excess dichromate solution with standard ferrous ammonium sulfate titrant, using the Ferroin indicator (3 drops) from blue-green to brown (Fig.2.13).

Calculation: Table 3.1. shows the results of COD-determination

TABLE 3.1. The results of COD-determination

Date, time	No sample	a (ml)	b (ml)	$mg/l\ COD = \frac{(a-b).N.8000}{ml\ sample}$
4 -2/1985	blank	17,30	-	
"	sample 1	-	12,48	

a- ml $Fe(NH_4)_2(SO_4)_2$ used for the blank,

b- ml $Fe(NH_4)_2(SO_4)_2$ used for the sample,

N- normality of $Fe(NH_4)_2(SO_4)_2$:

$$N = \frac{ml\ K_2Cr_2O_7 \times 0,25}{ml\ Fe(NH_4)_2(SO_4)_2}$$

Example : Take 10 ml $K_2Cr_2O_7$ 0,25N , 90 ml distilled water, 25 ml H_2SO_4 then titrate with standard ferrous ammonium sulfate $Fe(NH_4)_2(SO_4)_2$: 16,67 ml,

$$N = \frac{10 \times 0,25}{16,67} = 0,1498.$$

3.2. Sulphide (H_2S).

Photometric method of determination of sulpfide in water has been adapted after Trüper, H.G. and H.G.Schlegel; A.van Leeuwenhoek .

Principle : Dimethyn paraphenylene diaminesulphate reacts with sulphide and iron III to form methylene blue.

Reagents:

Zincacetate solution (ZnAc-solution),
Iron III ammonium sulphate solution ($FeNH_4(SO_4)_2$-solution),
DMP-solution ($H_2N-C_6H_4-N(CH_3).H_2SO_4$-solution).

Procedure

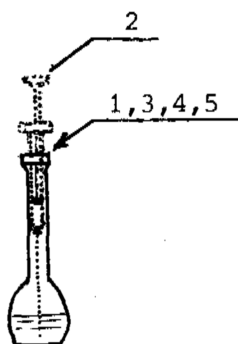


Fig.3.2. 50 ml graduated flask

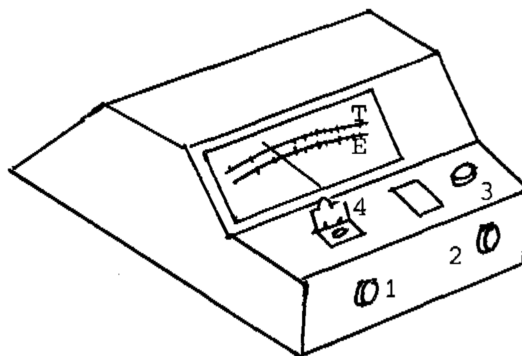


Fig.3.3.Spectrophotometer

1.pour 10 ml ZnAc-solution in a 50 ml graduated flask,
2.add the sample containing sulphide,below the liquid level (see Fig.3.2), (100 µl).Note : The sample volume should be chosen in order to keep the added amount of sulphide below 2 x 10^{-3} milimoles,
3.add 30 ml aqua dest.,
4.add 5 ml DMP-solution,
5.Swirl gently,
6.add 0,25 ml $FeNH_4(SO_4)_2$-solution immediately after 4 and 5,
7.swirl vigourously,
8.fill up to 50 ml with aqua dest.,
9.measure the Extinction at 670 nm against aqua dest.,after at least 15 minutes and at most 60 minutes(Fig.3.3).

Note with point 3 : After the sulphide has been fixed in the ZnAc-solution the sample can be kept in a refrigerator for some days.

Calculation :

The sulphide concentration can be calculated from a calibration-curve.This curve must be determined using solutions of a known concentration (for ex-example by iodometry).
(Calibration curve d.d. 17-7-1984 on Spectrophotometer No 8.76 at the Dep-Depart.of Water Polution Control,Wageningen)

$$[S] = (714{,}09 \times E + 10{,}83) \times \frac{50}{V_{sample}}$$

TABLE 3.2. H_2S concentration (mg/l)

time	Δt	No sample	Volume of sample µl	Extinction E	[S] mg/l
12-5					
14hr	-	No 1	100	0,028	15,41
17,30	3,5	No 2	100	0,026	14,70

3.3.Determination of the volatile fatty acids (VFA)

Two methods are used to determine the VFA:
a.titration method :

Apparatus

flask-250 ml,glass-150-200 ml,pipet 50 ml, filter-paper d=15 cm,pH-meter, titration equipment (Fig.2.13)...

Reagents : NaOH 0,1000N, HCl 0,1000N,boiling stone...

Procedure

1.Take 200 ml sample and fitrate by filter-paper d=15 cm (2 or 3 times) (Fig.3.4).
2.take 50 ml flitrated sample (by 50-pipet) and pour in the glass (Fig.3.5)

3.add 50 ml distilled water,add some boiling stones,

4.celebration of pH-meter with standard solution pH = 4 and pH = 7,

5.measure the pH,

6.titration of the sample with HCl 0,1000N up to pH=6,5,

7.then titrate the sample with also HCl 0,1000N up to pH=3 :
for example : ml HCl used for this titration: a= 13,73 (1^{rst} time,and
= 13,12 (2nd time)

8.pour the sample in COD-flask and attach the flask to the condenser and reflux for 3 min. (from boiling time),

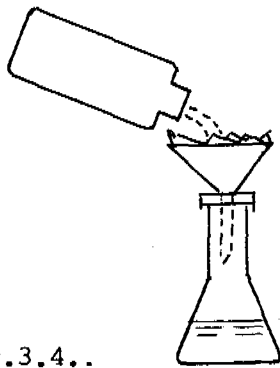


Fig.3.4..

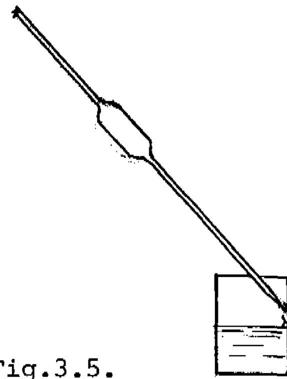


Fig.3.5.

9.cool for 2 min.,

10.then titrate the sample with NaOH 0,1000N up to pH=6,5 :
for example: ml NaOH used for this titration: b =2,79 (1^{rst} time and
=2,24 (2nd time)

Concentration VFA = C

$$C = \frac{b \times 101 - a - 100}{99,23} \quad , \text{meq/l}$$

TABLE 3.3. Total VFA-concentration

No sample	ml HCl a,ml	ml NaOH b,ml	C, meq/l
No 1	13,73	2,75	1,653
	13,12	2,24	1,140

b.gas chromatoghaph (Foto 2.1):

VFA-concenreation is done by gas chromatograph using a Becker Model 417 and computing integrator Model SP 4100. The small bottle for the sample is shown in Fig.2.24.The sample should be as clean as posible and can do it by mem - bran-filter.The Table 3.4 shows the results of VFA-concentration determina - tion by chromatograph (for eaxample):

TABLE 3.4. VFA determination by chromatograpg.

ANALYST : Lam thi Bao Minh

+++ MONSTERFLESJE 2 +++

NAME	mg/l	Org.C	COD	RT	BC	RF	RRT
C2	826,8	38.	881,9	1,06	02	702	-6 %
C3	2137,3	100.	3290,4	1,54	08	1180	x
i-C4	0,	0.	0,1	1,84	05	1565	-1,3 %
n-C4	31,3	1,4	56,8	2,33	05	1460	0,4 %

3.4. pH determination.

A Knick pH-meter (Fig.2.6) is used for pH measurements.

3.5. SO_4^{-2} determination.

SO_4^{-2} analysis are done by Autosampler Computing Integrator and HPLC ion chromatograph RI detector (Foto 2.2).The Table 3.5 shows the results of SO_4^{-2}determinations.

TABLE 3.5. SO_4^{-2} concentration.

ANALYST : Lam minh Tuan

NAME	conc.	RT	BC	RF	RRT
Chloride	533,344	4,5	01	13276,529	1
Sulphate	482,981	8,12	21	10595,754	1,804

Foto 2.1. Gas chromatograph (for VFA determination).

Foto 2.2.Autosampler Computing Integrator and HPLC ion chromatograph RI detector (For SO_4^{-2} determination).

3.6.The determination of ammonia (NH_4^+-N)

This standard is applicable for water containing 5 mg/l of NH_4^+-N at a maximum.

By applying a predistillation the interference by substances which produce a precipitate with Nessler's reagent ,is avoided.These substances are e.g. Ca^{2+},Mg^{2+},Fe,Al.

The interference by organic compounds like amines,aldehydes .alcohols etc. is not always completely eliminated.The interference by sulphides can be avoided by adding a little leadhydrocarbonate before the predistillation.

Principle

Ammonia forms a yellow-brown complex of ammonia-mercury-iodide ($HgNH_2I.HgO$) on addition of potassiummercuryiodide(Nessler's reagent) to the ammonia containing solution.

The extinction (E) of the coloured compound is measured at a wavelenght of 440 nm.

Reagents

-0.1 m Hydrochloric acid(HCl),

-Water,free from ammonia.If necessary redistill 1 litre of distilled water after addition of 1 ml of Sulfuric acid(H_2SO_4,18 m).

-Phosphate buffer pH = 7.3,

Dissolve 14.3 gr KH_2PO_4 and 90.2 gr $K_2HPO_4.3H_2O$ in water and dilute to 1 litre.

-Nessler's reagent,

Dissolve 100 gr of mercury(II)iodide and 70 gr of potassiumiodide in a small amount of water.

Pour this solution slowly and under stirring into a cooled solution of 150 gr of NaOH in 500 ml of water.Dilute to 1 litre.Keep the reagent in a polyethylene bottle in a dark place.

-Leadhydrocarbonate(or leadcarbonate).

Apparatus

A distillation apparatus,by preference completely made of glass,

A fotometer,suitable for measurements at approx. 440 nm ,and cuvettes (1 cm or 3.4 cm).

Sample

Start from a sample of 250 ml (for analysis of water with ammonia concentration lower than 5 mg/l).If the ammonia-concentration is higher start from a smaller sample (e.g. 100 ml).
If necessary the sample should be neurtalised.

Procedure

Add to the flask which has to receive the distillate 5 ml HCl and 25 ml water.Connect the flask to the distillaltion apparatus.
Add the sample to the distillation flask.Add some glass pearls,some leadhydrocarbonate(leadcorbonate) and 25 ml phosphate buffer.Connect this flask to the apparatus immediately.
Then distill until the half of the sample has passed to the receiving flask.Transfer the distillate to a volumetric flask of 250 ml,dilute and shake.This flask now contains all the ammonia present.
Add a suitable amount of the distillate from this flask (v ml which contains 0.02 - 0.4 mg ammonia) to a volumetric flask of 100 ml.
Dilute to 90 ml with ammonia free water.Add 2 ml of Nessler's reagent, dilute and mix.
Determine the extinction at a wavelength of 440 nm and a pathlength of 1 of 3.4 cm.
Also determine the extinction of the blank.

The calibration curve

Dissolve 3.818 gr NH_4Cl in water in a volumetric flask of 1 litre, dilute and mix.Dilute this stock solution 1:100.
1 ml of this solution corresponds to 0.01 mg NH_4^+ -N.
Add 2-5-10-15-20 ml of this diluted solution to volumetric flask of 100 ml.
Dilute to 90 ml and proceed as described above.
Make a graph of the extinction ($E - E_o$) as a function of the amounts of NH_4^+-N.

Calculation

Calculate the NH_4^+-N concentration in the sample from the calibration curve.

3.7. Total Nonfiltrable Residue Dried at 103-105 C (Total Suspended Matter)

1. General Discussion

Total nonfiltrable residue is the retained material on a standard glass fiber filter disk after filtration of a well mixed sample of water or wastewater. The residue is dried at 103 to 105 C. If the suspended material clogs the filter and prolongs the filtration time, the difference between the total residue and the total filtrable residue provides an estimate of the total nonfiltrable residue.

2. Apparatus

Apparatus listed in Sections 208 A.2 and 208 B.2 is required.

3. Procedure

a. Preparation of glass fiber filter disk: Place the disk either on the membrane filter apparatus or the bottom of a suitable Gooch crucible. Apply vacuum and wash the disk with three successive 20-ml portions of distilled water. Continue suction to remove all traces of water from the disk, and discard the washings. Remove the filter from the membrane filter apparatus and transfer to an aluminum or stainless steel planchet as a support. Remove the crucible and filter combination if a Gooch crucible is used. Dry in an oven at 103 to 105 C for 1 hr. Store in desiccator until needed. Weigh immediately before use.

b. Sample treatment: Since excessive residue on the filter may entrap water and extend the drying time, take for analysis a sample that will yield no more than 200 mg total nonfiltrable residue. Under vacuum, filter 100 ml (or a larger volume if total nonfiltrable residue is low) well mixed sample. Carefully remove the filter from the membrane filter funnel assembly, and transfer to an aluminum or stainless steel planchet as a support. Remove the crucible and filter combination from crucible adapter if a Gooch crucible is used. Dry for at least 1 hr at 103 to 105 C, cool in a desiccator, and weigh. Repeat the drying cycle until a constant weight is attained or until weight loss is less than 0.5 mg.

4. Calculation

$$\text{mg/l total nonfiltrable residue} = \frac{(A-B)\times 1{,}000}{\text{ml sample}}$$

where A = weight of filter + residue and B = weight of filter.

5. Precision and Accuracy

The precision of the determination varies directly with the concentration of suspended matter in the sample. The standard deviation was ±5.2 mg/l (coefficient of variation 33%) at 15 mg/l, ±24 mg/l (10%) at 242 mg/l, and ±13 mg/l (0.76%) at 1,707 mg/l (n = 2; 4×10). There is no satisfactory procedure for obtaining the accuracy of the method on wastewater samples, because the true concentration of suspended matter is unknown. See Section 208A.5 for other comments.

3.8. OXYGEN DEMAND (BIOCHEMICAL)*

1. Discussion

The biochemical oxygen demand (BOD) determination described herein is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen requirements of wastewaters, effluents, and polluted waters. The test has its widest application in measuring waste loadings to treatment plants and in evaluating the efficiency (BOD removal) of such treatment systems. BOD values cannot be compared unless the results have been obtained under identical test conditions.

The test is of limited value in measuring the actual oxygen demand of surface waters. The extrapolation of test results to actual stream oxygen demands is highly questionable because the laboratory environment does not reproduce stream conditions such as temperature, sunlight, biological population, water movement, and oxygen concentration.

Samples for BOD analysis may undergo significant degradation during handling and storage. Some of the demand may be satisfied if the sample is held for several days before the test is initiated; this results in a low estimation of the true BOD. The extent of change appears to be a function of the amount of organic matter (food supply) and the number and types of organisms (biological population). To reduce the change in oxygen demand that occurs between sampling and testing, keep all samples at or below 4 C and begin incubation not more than 24 hr after the sample is collected.

The amount of oxygen demand in the sample will govern the need for and the degree of dilution.

Aerate samples with low DO values to increase the initial DO content above that required by the BOD. Let air bubble through a diffusion tube into the sample for 5 min, or until the DO is at least 7 mg/l. Determine DO on one portion of the aerated sample; seed another portion only if necessary, and incubate it for the BOD determination.

Complete stabilization of a given waste may require a period of incubation too long for practical purposes. For this reason, the 5-day period has been accepted as standard. However, for certain industrial wastes it may be advisable to determine the oxidation curve obtained. Conversion of data from one incubation period to another can be made only if such special studies are carried out. Studies in recent years have shown that the exponential rate of carbonaceous oxidation, k, at 20 C rarely has a value of 0.1, although it may vary from less than one-half to more than twice this value. This fact usually makes it impossible to calculate the ultimate carbonaceous demand, L, of a sample from 5-day BOD values unless the k value has been determined on the sample. The exponential interpretation of BOD rate curves is a gross oversimplification; a good exponential fit is not obtained always.

The test measures the oxygen demand produced by carbonaceous and nitrogenous compounds, and immediate oxidation. All of these have a bearing on the oxygen balance of the receiving water and must be considered in the discharge of a waste to such water. Differentiation of the immediate dissolved oxygen demand is described in ¶4*j* below. Appropriate technics for the suppression of nitrification in tests for carbonaceous demand only are given elsewhere.[1-5] If nitrification suppression is used, state this clearly when reporting results. Bear in mind that some suppressors may also inhibit carbonaceous oxidation.

*BOD, biochemical oxygen demand.

2. Apparatus

a. Incubation bottles, 250- to 300-ml capacity, with ground-glass stoppers. Clean bottles with a good detergent, rinse thoroughly, and drain before use. As a precaution against drawing air into the dilution bottle during incubation, use a water seal. Satisfactory water seals are obtained by inverting the bottles in a water bath or adding water to the flared mouth of special BOD bottles.

b. Air incubator or water bath, thermostatically controlled at 20 C±1 C: Exclude all light to prevent formation of DO by algae in the sample.

3. Reagents

a. Distilled water: Use only high-quality water distilled from a block tin or all-glass still. Alternatively, use de-

ionized water. The water must contain less than 0.01 mg/l copper, and be free of chlorine, chloramines, caustic alkalinity, organic material, or acids.

b. Phosphate buffer solution: Dissolve 8.5 g potassium dihydrogen phosphate, KH_2PO_4; 21.75 g dipotassium hydrogen phosphate, K_2HPO_4; 33.4 g disodium hydrogen phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$; and 1.7 g ammonium chloride, NH_4Cl, in about 500 ml distilled water and dilute to 1 l. The pH of this buffer should be 7.2 without further adjustment. Discard the reagent (or any of the following reagents) if there is any sign of biological growth in the stock bottle.

c. Magnesium sulfate solution: Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 l.

d. Calcium chloride solution: Dissolve 27.5 g anhydrous $CaCl_2$ in distilled water and dilute to 1 l.

e. Ferric chloride solution: Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 l.

f. Acid and alkali solutions, 1N: For neutralization of caustic or acidic waste samples.

g. Sodium sulfite solution, 0.025N: Dissolve 1.575 g anhydrous Na_2SO_3 in 1,000 ml distilled water. This solution is not stable; prepare daily.

h. Seeding: The purpose of seeding is to introduce into the sample a biological population capable of oxidizing the organic matter in the wastewater. Where such microorganisms are already present, as in domestic wastewater or unchlorinated effluents and surface waters, seeding is unnecessary and should not be used.

When the sample contains very few microorganisms—as a result, for example, of chlorination, high temperature, or extreme pH—seed the dilution water. The standard seed material is settled domestic wastewater that has been stored at 20 C for 24 to 36 hr. Use sufficient seed to produce a seed correction (¶4g) of at least 0.6 mg/l.

Some samples—for example, certain industrial wastes—may require seeding because of low microbial population, but they contain organic compounds that are not readily oxidized by domestic wastewater seed. For evaluating the effect of such a waste in a treatment system, it is better to use specialized seed material containing organisms adapted to the use of the organic compounds present. Obtain such adapted seed from the effluent of a biological treatment process receiving the waste in question, or from the receiving water below the point of discharge [preferably 3 to 8 km (2 to 5 miles) below] if the waste is not being treated. When these sources are not available, develop adapted seed in the laboratory by continuously aerating a large sample of water and feeding it with small daily increments of the particular waste, together with soil or domestic sewage, until a satisfactory microbial population has developed. The special circumstances that call for the use of adapted seed also may require a seed concentration higher than the standard 1 to 2 ml/l. Decide on the kind and amount of seed required for such special-purpose studies on the basis of prior experience with the particular waste and the purpose for which the determination is being made.

Adapted seed also has been used in attempts to estimate the effect of a waste on the receiving water. (See Section 507.1).

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4. Procedure

a. Preparation of dilution water: Before use, store the distilled water in cotton-plugged bottles long enough for it to become saturated with DO; or, if such storage is not practical, saturate the water by shaking the partially filled bottle or by aerating with a supply of clean compressed air. Use distilled water at 20±1 C.

Place the desired volume of distilled water in a suitable bottle and add 1 ml each of phosphate buffer, $MgSO_4$, $CaCl_2$, and $FeCl_3$ solutions/l of water. If dilution water is to be stored in the incubator, add the phosphate buffer just before using the dilution water.

b. Seeding: See ¶ 3*b* et seq, preceding. If the dilution water is seeded, use it the same day it is prepared.

c. Pretreatment:

1) Samples containing caustic alkalinity or acidity—Neutralize to about pH 7.0 with 1*N* H_2SO_4 or NaOH, using a pH meter or bromthymol blue as an outside indicator. The pH of the seeded dilution water should not be changed by the preparation of the lowest dilution of sample.

2) Samples containing residual chlorine compounds—If the samples stand for 1 to 2 hr, the residual chlorine often will be dissipated. Prepare BOD dilutions with properly seeded standard dilution water. Destroy higher chlorine residuals in neutralized samples by adding Na_2SO_3. Determine the appropriate quantity of sodium sulfite solution on a 100- to 1,000-ml portion of the sample by adding 10 ml of 1+1 acetic acid or 1+50 H_2SO_4, followed by 10 ml KI solution (10 g/100 ml) and titrating with 0.025*N* Na_2SO_3 solution to the starch-iodide end point. Add to a volume of sample the quantity of Na_2SO_3 solution determined by the above test, mix, and after 10 to 20 min test a sample for residual chlorine to check the treatment. Prepare BOD dilutions with seeded standard dilution water.

3) Samples containing other toxic substances—Samples such as those from industrial wastes—for example, toxic metals derived from plating wastes—frequently require special study and treatment.

4) Samples supersaturated with DO—Samples containing more than 9 mg/l DO at 20 C may be encountered during winter months or in localities where algae are growing actively. To prevent loss of oxygen during incubation of these samples, reduce the DO to saturation by bringing the sample to about 20 C in a partly filled bottle and agitating it by vigorous shaking or by aerating with compressed air.

d. Dilution technic: Make several dilutions of the prepared sample to obtain the required depletions. The following dilutions are suggested: 0.1 to 1.0% for strong trade wastes, 1 to 5% for raw and settled sewage, 5 to 25% for oxidized effluents, and 25 to 100% for polluted river waters.

1) Carefully siphon standard dilution water, seeded if necessary, into a graduated cylinder of 1,000 to 2,000 ml capacity, filling the cylinder half full without entrainment of air. Add the quantity of carefully mixed sample to make the desired dilution and dilute to the appropriate level with dilution water. Mix well with a plunger-type mixing rod, avoiding entrainment of air. Siphon the mixed dilution into two BOD bottles, one for incubation and the other for de-

termination of the initial DO in the mixture; stopper tightly and incubate for 5 days at 20 C. Water-seal the BOD bottles by inverting in a tray of water in the incubator or by using a special water-seal bottle. Prepare succeeding dilutions of lower concentration in the same manner or by adding dilution water to the unused portion of the preceding dilution.

2) The dilution technic may be greatly simplified when suitable amounts of sample are measured directly into bottles of known capacity with a large-tip volumetric pipet and the bottles are filled with sufficient dilution water to permit insertion of the stopper without leaving air bubbles. Make dilutions greater than 1:100 by diluting the waste in a volumetric flask before adding it to the incubation bottles for final dilution.

e. Determination of DO: If the sample represents 1% or more of the lowest BOD dilution, determine DO on the undiluted sample. This determination is usually omitted on sewage and settled effluents known to have a DO content of practically zero. ***With samples having an immediate oxygen demand, use a calculated initial DO, inasmuch as such a demand represents a load on the receiving water.***

f. Incubation: Incubate the blank dilution water and the diluted samples for 5 days in the dark at 20 C. Then determine the DO in the incubated samples and the blank using the azide modification of the iodometric method or a membrane electrode. Unless the membrane electrode is used, use the alum flocculation method for incubated samples of muds and the copper sulfate-sulfamic acid method for activated sludges. In special cases, other modifications may be necessary. Those dilutions showing a residual DO of at least 1 mg/l and a depletion of at least 2 mg/l are most reliable.

g. Seed correction: If the dilution water is seeded, determine the oxygen depletion of the seed by setting up a separate series of seed dilutions and selecting those resulting in 40 to 70% oxygen depletions in 5 days. Use one of these depletions to calculate the correction due to the small amount of seed in the dilution water. Do not use the seeded blank for seed correction because the 5-day seeded dilution water blank is subject to erratic oxidation due to the very high dilution of seed, which is not characteristic of the seeded sample.

h. Dilution water control: Fill two BOD bottles with unseeded dilution water. Stopper and water-seal one of these for incubation. Determine the DO before incubation in the other bottle. Use the DO results on these two bottles as a rough check on the quality of the unseeded dilution water. Do not use the depletion obtained as a blank correction; it should not be more than 0.2 mg/l and preferably not more than 0.1 mg/l.

i. Glucose-glutamic acid check: The BOD test is a bioassay procedure; consequently, the results obtained are influenced greatly by the presence of toxic substances or the use of a poor seeding material. Distilled waters frequently are contaminated with toxic substances—most often copper—and some sewage seeds are relatively inactive. The results obtained with such waters are always low.

The quality of the dilution water, the effectiveness of the seed, and the technic of the analyst should be checked periodically by using pure organic compounds

having known or determinable BOD. If a particular organic compound is known to be present in a given waste, it may well serve as a control on the seed used. For general BOD work, a mixture of glucose and glutamic acid (150 mg/l of each) has certain advantages. Glucose has an exceptionally high and variable oxidation rate with relatively simple seeds. When it is used with glutamic acid, the oxidation rate is stabilized and is similar to that obtained with many municipal wastes (0.16 to 0.19 exponential rate). In exceptional cases, a given component of a particular waste may be the best choice to test the efficacy of a particular seed.

To check the dilution water, the seed material, and the technic of the analyst, prepare a standard solution containing 150 mg/l each of reagent-grade glucose and glutamic acid that have been dried at 103 C for 1 hr. Pipet 5.0 ml of this solution into calibrated incubation bottles, fill with seeded dilution water, and incubate with seed control at 20 C for 5 days. On the basis of a mixed primary standard containing 150 mg/l each of glucose and glutamic acid, the 5-day BOD varies in magnitude according to the type of seed, and precision varies with the quality of seed, as shown in Table 507:I.

Except with the oxidized river water and effluents, a low seed correction resulted in an appreciably higher value for the standard deviation. Check each seed source to determine the amount required to obtain optimum precision. If results differ appreciably from those given in Table 507:I after the seed source has been considered, the technic is questionable.

j. Immediate dissolved oxygen demand: Substances oxidizable by molecular oxygen, such as ferrous iron, sulfite, sulfide, and aldehyde, impose a load on the receiving water and must be taken into consideration. The total oxygen demand of such a substrate may be determined by using a calculated initial DO or by using the sum of the immediate dissolved oxygen demand (IDOD) and the 5-day BOD. Where a differentiation of the two components is desired, determine the IDOD. The IDOD does not necessarily represent the immediate oxidation by molecular DO but may represent an oxidation by the iodine liberated in the acidification step of the iodometric method.

TABLE 507:I. EFFECT OF SEED TYPE AND QUALITY ON BOD RESULTS

Type of Seed	5-day Seed Correction *mg/l*	Mean 5-day BOD *mg/l*	Standard Deviation *mg/l*
Settled fresh sewage	>0.6	218	±11
Settled stale sewage	>0.6	207	± 8
River water (4 sources)	0.05-0.22	224-242	±7-13
Activated sludge effluent	0.07-0.68	221	±13
Trickling filter effluent	0.2-0.4	225	± 8

The depletion of DO in a standard water dilution of the sample in 15 min has been arbitrarily selected as the IDOD. To determine the IDOD, separately measure the DO of the sample (which in most cases is zero) and the DO of the dilution water. Prepare an appropriate dilution of the sample and dilution water and determine the DO after 15 min. The calculated DO of the sample dilution minus the observed DO after 15 min is the IDOD, in milligrams per liter, of the sample dilution.

5. Calculation

a. Definitions:

D_0 = DO of original dilution water
D_1 = DO of diluted sample 15 min after preparation
D_2 = DO of diluted sample after incubation
S = DO of original undiluted sample
D_c = DO available in dilution at zero time
= $D_0 p + SP$
p = decimal fraction of dilution water used
P = decimal fraction of sample used
B_1 = DO of dilution of seed control before incubation
B_2 = DO of dilution of seed control after incubation
f = ratio of seed in sample to seed in control

$$= \frac{\%\ \text{seed in } D_1}{\%\ \text{seed in } B_1}$$

Seed correction $= (B_1 - B_2)f$.

b. Biochemical oxygen demand:
When seeding is not required,

$$\text{mg/l BOD} = \frac{D_1 - D_2}{P}$$

When using seeded dilution water,

$$\text{mg/l BOD} = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

Including IDOD if small or not determined,

$$\text{mg/l BOD} = \frac{D_c - D_2}{P}$$

c. Immediate dissolved oxygen demand:

$$\text{mg/l IDOD} = \frac{D_c - D_1}{P}$$

The DO determined on the unseeded dilution water after incubation is not used in the BOD calculations because this practice would overcorrect for the dilution water. In all the above calculations, corrections are not made for small losses of DO in the dilution water during incubation. If the dilution water is unsatisfactory, proper corrections are difficult and the results are questionable.

6. Precision and Accuracy

At present there is no standard against which the accuracy of the BOD test can be measured. To obtain interlaboratory precision data, a glucose-glutamic acid mixture (¶4*i* preceding) with a theoretical oxygen demand value of 194 mg/l was analyzed by 73 participants, with each laboratory using its own seed material. The arithmetic mean of all results was 175 mg/l and the standard deviation of that mean was ±26 mg/l (15%).

7. References

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3.9. STANDARD PLATE COUNT

1. Introduction

The Standard Plate Count procedure provides a standardized means of determining the density of aerobic and facultative anaerobic heterotrophic bacteria in water. This is an empirical measurement because bacteria occur singly, in pairs, chains, clusters, or packets, and no single growth medium or set of physical and chemical conditions can satisfy the physiological requirements of all bacteria in a water sample. Consequently, the number of colonies may be lower substantially than the actual number of viable bacteria present. To facilitate the collection of reliable data for water quality control measurements, especially for comparative and legal purposes, a standardized plate count procedure is essential.

2. Work Area

A level table or bench top with ample area should be available in a clean, draft-free, well-lighted room. Table and bench tops should have a nonporous surface and should be disinfected before any analysis is conducted.

3. Samples

Potable water samples from a distribution system should be collected as directed in Section 906A. Initiate sample analysis as soon as possible to minimize changes in the bacterial population. The recommended maximum elapsed time between collection and examination of unrefrigerated samples is 8 hr (maximum transit time 6 hr, maximum processing time 2 hr). When analysis cannot begin within 8 hr, maintain the sample at a temperature below 10 C. The maximum elapsed time between collection and analysis shall not exceed 30 hr.

Bottled water samples obtained from retail outlets may be held or transported unrefrigerated provided the temperature does not exceed 20 to 25 C. Examine freshly bottled samples (less than 48 hr old) within 6 hr of collection if unrefrigerated and within 30 hr if refrigerated.

4. Sample Preparation

Mark each plate with sample number, dilution, date, and any other necessary information before sample examination. Prepare duplicate plates for each volume of sample or sample dilution examined.

Thoroughly mix all samples by making 25 complete up-and-down (or back-and-forth) movements of about 0.3 m (1 ft) in 7 sec. Optionally, use a mechanical shaker to shake the dilution blanks for 15 sec.

5. Sample Dilution

Prepare water used for dilution blanks as directed in Media Specifications, Section 905 C.

a. Selecting dilutions: Select the dilution(s) so that the total number of colonies on a plate will be between 30 and 300 (Figure 907.1). For example, where a Standard Plate Count as high as 3,000 may be suspected, prepare plates containing 1:100 dilution.

For most potable water samples, plates suitable for counting will be obtained by planting 1 ml and 0.1 ml of undiluted sample and 1 ml of sample diluted 1:100.

b. Measuring sample portions: Use a sterile pipet for initial and subsequent transfers from each container. If the pipet becomes contaminated before the transfers are completed, replace it with a sterile pipet. Use a separate sterile pipet for transfers from each different dilution. Do not prepare dilutions and pour plates in direct sunlight. Use caution when removing sterile pipets from the container; to avoid contamination of the pipet, do not drag the tip across the exposed ends of pipets or across the lips and necks of dilution bottles. When removing sample, do not insert pipets more than 2.5 cm (1 in.) below the surface of the sample or dilution.

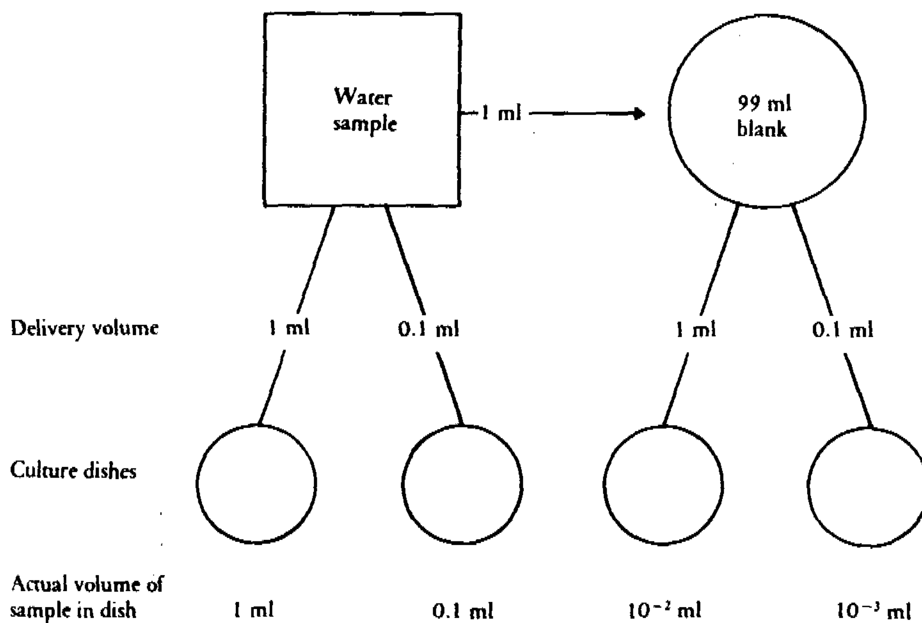

Figure 907:1. Preparation of dilutions.

c. Measuring dilutions: When measuring diluted samples of water, hold the pipet at an angle of about 45° with the tip touching the inside cover of the petri dish or the inside neck of the dilution bottle. Lift the cover of the petri dish just

high enough to insert the pipet. Allow 2 to 4 sec for the liquid to drain from the 1 ml graduation mark to the tip of the pipet. If the pipet is not a blow-out type, touch the tip of the pipet *once* against a dry spot in the petri plate. If the pipet is, less preferably, a blow-out type, it must have a cotton plug in the mouthpiece; gently blow out the remaining volume of sample dilution. When 0.1-ml quantities are measured, let the diluted sample drain from the chosen reference graduation until 0.1 ml has been delivered. Remove the pipet without retouching it to the plate. Pipet 1 ml, 0.1 ml, or other suitable volume of the dilution to be used for plating in the sterile petri dish before adding the melted culture medium. It is recommended that decimal dilutions be used in preparing sample volumes of less than 1 ml; in the examination of sewage or turbid water, do not measure a 0.1-ml inoculum of the original sample, but prepare an appropriate dilution. Prepare at least two replicate plates for each sample dilution used. After depositing test portions for each series of plates, pour the culture medium.

6. Plating

a. Melting medium: Melt sterile solid agar medium in boiling water or by exposure to flowing steam in a partially closed container, but avoid prolonged exposure to unnecessarily high temperatures during and after melting. Do not resterilize the plating medium. If the medium is melted in two or more batches, use all of each batch in order of melting, provided that the contents in separate containers remain fully melted. Discard melted agar that contains precipitate.

Temper the melted medium in a water bath between 44 C and 46 C until used. In a separate container place a thermometer in water or medium that has been exposed to the same heating and cooling as the plating medium. Do not depend on the sense of touch to indicate the proper temperature of the medium when pouring agar.

Use tryptone glucose extract agar or plate count agar, as specified in Section 905 C.

b. Pouring the plates: Limit the number of samples to be plated in any one series so that no more than 20 min (preferably 10 min) elapse between dilution of the first sample and pouring of the last plate in the series. Pour at least 10 to 12 ml of liquefied medium at 44 to 46 C into each plate by gently lifting the cover of the petri dish just high enough to pour the medium. Carefully avoid spilling the medium on the outside of the container or on the inside of the plate lid when pouring. As each plate is poured mix the melted medium thoroughly with the test portions in the petri dish, taking care not to splash mixture over the edge, by rotating the dish first in one direction and then in the opposite direction, or by rotating and tilting the dish. Allow the plates to solidify (within 10 min) on a level surface. After the medium solidifies, invert the plates and place them in the incubator.

c. Sterility controls: Check the sterility of the medium and the dilution water blanks by pouring control plates for each series of samples. Additional controls also may be prepared to determine contamination of plates, pipets, and room air.

7. Incubation

Incubate for the Standard Plate Count for all water samples except bottled water at a temperature of 35±0.5 C for 48±3 hr.

For the Standard Plate Count of bottled water, the plates shall be incubated at 35±0.5 C for 72±4 hr. Since many of the bacteria found in bottled water demonstrate a prolonged lag phase during adaptation to growth on tryptone glucose extract agar or plate count agar, such bacteria do not form colonies that can be counted after 48 hr incubation so that an additional 24 hr incubation is required to obtain a reliable Standard Plate Count.

Plates should be packed as directed under Laboratory Apparatus, Section 903, without crowding in the incubator. Any deviation from this method must be stated in the examination report.

8. Counting and Recording

Count all colonies on selected plates promptly after the incubation period. If counting must be delayed temporarily, store plates at 5 to 10 C for a period of no more than 24 hr, but avoid this as routine practice. Record the results of sterility controls on the report for each lot of samples.

Use an approved counting aid, such as the Quebec colony counter, for manual counting. If such equipment is not available, counting may be done with any other counter provided that it gives equivalent magnification and illumination. Automatic plate counting instruments are now available. These generally use a television scanner coupled to a magnifying lens and an electronics package. Their use is acceptable if evaluation in parallel with manual counting gives comparable results.

In preparing plates, volumes of sample should be planted that will give from 30 to 300 colonies on a plate. The aim should be to have at least one dilution for which the replicate plates give colony counts between these limits, except as provided below.

Ordinarily, it is not desirable to plant more than 1.0 ml of water in a plate; therefore, when the total number of colonies developing from 1.0 ml is less than 30, it is necessary to disregard the rule above and record the result as observed. With this exception, only plates showing 30 to 300 colonies should be considered in determining the Standard Plate Count. Compute the bacterial count per milliliter by multiplying the average number of colonies per plate by the dilution used. Report as the "Standard Plate Count" per milliliter.

If there is no plate with 30 to 300 colonies, and one or more plates have more than 300 colonies, use the plate(s) having a count nearest 300 colonies. Compute the count by multiplying the average count per plate by the dilution used and report as the "Estimated Standard Plate Count" per milliliter.

If plates from all dilutions of any sample have no colonies, report the count as less than one (<1) times the corresponding lowest dilution. For example, if no colonies develop on the 1:100 dilution, report the count as "less than 100 (<100) Estimated Standard Plate Count" per milliliter.

If the number of colonies per plate far exceeds 300, do not report the result as "too numerous to count" (TNTC). If there are fewer than 10 colonies/cm^2, count colonies in 13 squares (of the col-

ony counter) having representative colony distribution. If possible, select seven consecutive squares horizontally across the plate and six consecutive squares at right angles, being careful not to count a square more than once. Multiply the sum of the colonies in 13 representative cm^2 by 5 to compute the estimated colonies per plate when the area of the plate is 65 cm^2. When there are more than 10 colonies/cm^2, count four representative squares, take the average count per square cm and multiply by the appropriate factor to estimate the colonies per plate (usually about 65). When bacterial counts on crowded plates are greater than 100 colonies/cm^2, report the result as greater than (>) 6,500 times the highest dilution plated.

If spreading colonies (spreaders) are encountered on the plate(s) selected, count colonies on representative portions only when (*a*) colonies are well distributed in spreader-free areas, and (*b*) the area covered by the spreader(s) does not exceed one-half the plate area.

When spreading colonies must be counted, count each unit of the following types as one: (*a*) The first is a chain of colonies that appears to be caused by disintegration of a bacterial clump as the agar and sample were mixed. Count each such chain as a single colony, do not count each individual colony in the chain; (*b*) The second type of spreader develops as a film of growth between the agar and the bottom of the petri dish; (*c*) The third type forms in a film of water at the edge or over the surface of the agar. Types *b* and *c* largely develop because of an accumulation of moisture at the point from which the spreader originates. They frequently cover more than half the plate and interfere with obtaining a reliable plate count.

If plates prepared from the samples have excessive spreader growth, report as "Spreaders" (Spr). When plates are uncountable because of missed dilution, accidental dropping, and contamination, or the control plates indicate that the medium or other material or labware was contaminated, report as "Laboratory Accident" (LA).

9. Computing and Recording Counts

To compute the Standard Plate Count, multiply the total number of colonies or the average number (if duplicate plates of the same dilution) per plate by the reciprocal of the dilution used. Record the dilutions used and the number of colonies on each plate counted or estimated.

When colonies on duplicate plates and/or consecutive dilutions are counted and the results are averaged before being recorded, round off counts to two significant figures only at the time of conversion to the Standard Plate Count.

Avoid creating fictitious ideas of precision and accuracy when computing Standard Plate Counts, by recording only the first two left-hand digits. Raise the second digit to the next highest number only when the third digit from the left is 5, 6, 7, 8, or 9; use zeros for each successive digit toward the right from the second digit. For example, a count of 142 is recorded as 140, and a count of 155 as 160, whereas a count of 35 is recorded as 35.

10. Reporting Counts

Report counts as "Standard Plate Count" or "Estimated Standard Plate Count" per milliliter.

11. Personal Errors

Avoid inaccuracies in counting due to carelessness, damaged or dirty optics that impair vision, or failure to recognize colonies. Laboratory workers who cannot duplicate their own counts on the same plate within 5% and the counts of other analysts with 10%, should discover the cause and correct such disagreements.

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3210·MULTIPLE-TUBE FERMENTATION TECHNIC FOR MEMBERS OF THE COLIFORM GROUP

The coliform group comprises all of the aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas formation within 48 hr at 35 C.*

The standard test for the coliform group may be carried out either by the multiple-tube fermentation technic (presumptive test, confirmed test, or completed test) described herein or by the membrane filter technic described under a separate heading, each technic being applicable within the limitations specified and with due consideration of the purpose of the examination.

As applied to the membrane filter technic, the coliform group may be redefined as comprising all the aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that produce a dark colony with a metallic sheen within 24 hr on an Endo-type medium containing lactose.

It has been adequately demonstrated that, even after the prescribed shaking,

* The "coliform group" as defined above is equivalent to the "*B. coli* group" as used in the third, fourth and fifth editions of this manual, and to the "*coli-aerogenes* group" as used through the eighth edition.

the distribution of bacteria in water is irregular. It is entirely possible to divide a given volume of water into portions and after testing find that the number of organisms in any portion may be none, or at least less than the arithmetic average based on examination of the total volume might indicate. It is also quite probable that the growth in a fermentation tube may result not from one organism but from many organisms. It is reasonable, however, to assume that growth develops from a single individual.

It is convenient to express the results of the examination of replicate tubes and dilutions in terms of the Most Probable Number (MPN). This term is actually an estimate based on certain probability formulas. Theoretical considerations and large-scale replicate determinations indicate that this estimate tends to be greater than the actual number and that the disparity tends to diminish with increasing numbers of tubes in each dilution examined.

The accuracy of any single test will depend, then, on the number of tubes used. The most satisfactory information will be obtained when the largest portion examined shows gas in some or all of the tubes and the smallest portion shows no gas in all or a majority of the tubes. The numerical value of the estimation of the bacterial content is determined largely by the dilution that shows both positive and negative results. The number of portions scheduled, especially in the critical dilution, will be governed by the desired accuracy of the result. The increased interest in the multiple-tube technic, the numerous investigations into its precision, and the expression of test results as MPNs should not lead the analyst to regard this method as a statistical exercise rather than a means of estimating the coliform density of a water and thereby an aid to establishing its sanitary quality. The best assessment of the sanitary quality of a water still must depend on the interpretation of results of the multiple-tube technic—or of other methods, possibly more precise—and of all other information regarding a water that may be obtained by surveys or otherwise.

1. Water of Drinking Water Quality

When water is examined for evidence of quality that meets the standards of the U.S. Environmental Protection Agency, it is necessary to use five fermentation tubes of the presumptive medium, each containing 10 ml or 100 ml of the water sample. Practical considerations generally militate against the use of larger portions. The Confirmed Test or the Completed Test shall be the test of choice.

For water examined frequently, or even daily, the common practice of inoculating five 10-ml or five 100-ml portions generally provides sufficient definite information. In the examination of other waters presumed to be of drinking-water quality, the use of at least five tubes in each of at least three dilutions is desirable to provide acceptable precision and reasonably satisfactory information. In no case should less than three tubes per dilution be used.

For the routine examination of most potable water supplies, particularly those that are disinfected, the object of the test is to determine the presence or absence of coliform organisms as a

3.11. Standard Total Coliform MPN Tests

1. Presumptive Test

Lactose broth or lauryl tryptose broth may be used in the Presumptive Test.

a. Procedure:

1) Inoculate a series of fermentation tubes ("primary" fermentation tubes) with appropriate graduated quantities (multiples and submultiples of 1 ml) of the water to be tested. Bottles to contain 100-ml sample portions should be prewarmed in a water bath at 35 C; after adding the sample mix thoroughly and aseptically add a sterile fermentation vial. The concentration of nutritive ingredients in the mixture of medium and added portion of sample must conform to the requirements given in Section 905C, Media Specifications, Media 2 and 3. The portions of the water sample used for inoculating the lactose or lauryl tryptose broth fermentation tubes will vary in size and number with the character of the water under examination, but in general should be decimal multiples and submultiples of 1 ml. These should be selected in accordance with the discussion of the multiple-tube test above.

2) Incubate the inoculated fermentation tubes at 35±0.5C. At the end of 24±2 hr, shake each tube gently and examine it and, if no gas has formed and been trapped in the inverted vial, repeat this step at the end of 48±3 hr. Record the presence or absence of gas formation at each examination of the tubes, regardless of the amount.

b. Interpretation: Formation within 48±3 hr of gas in any amount in the inner fermentation tubes or vials constitutes a positive Presumptive Test.

The appearance of an air bubble must not be confused with actual gas production. If the gas is formed as a result of fermentation, the broth medium will become cloudy. *Active fermentation* may be shown by the continued appearance of small bubbles of gas throughout the medium outside the inner vial when the fermentation tube is gently shaken.

The absence of gas formation at the end of 48±3 hr of incubation constitutes a *negative test*. An arbitrary limit of 48 hr for observation doubtless excludes from consideration occasional members of the coliform group that form gas very slowly and are generally of limited sanitary significance; for the purpose of a standard test based on the definition of the coliform group, exclusion of these occasional slow gas-forming organisms does not compromise the value of the test.

2. Confirmed Test

Lactose broth or lauryl tryptose broth may be used for the primary fermentation; however, lauryl tryptose broth is recommended when experience shows a high proportion of false positive tubes of lactose broth.

Use brilliant green lactose bile broth fermentation tubes for the Confirmed Test.

a. Procedure: Submit all primary fermentation tubes showing any amount of gas at the end of 24 hr of incubation to the confirmed test. If active fermentation appears in the primary fermentation tube before expiration of the 24-hr period of incubation, it is preferable to transfer to the confirmatory medium without waiting for the full 24-hr period to elapse. If additional primary fermentation tubes show gas production at the

end of 48-hr incubation, these too shall be submitted to the confirmed test.

b. Alternative procedure: Where three or more multiple portions of a series of three or more decimal dilutions of a given sample are planted, submit to the Confirmed Test all tubes of the two highest dilutions (smallest volumes) of the original samples showing gas formation in 24 hr.

All tubes producing gas in 24 hr that have not been submitted to the Confirmed Test must be recorded as containing organisms of the coliform group—that is, as positive—even though all the confirmed tests actually performed yield negative results.

Submit to the Confirmed Test all tubes of all dilutions of the original sample in which gas is produced only at the end of 48 hr.

If less than three portions of any dilution (volume), or if a series of less than three decimal dilutions of the original sample is planted, submit all tubes producing gas at 24 or 48 hr to the confirmed test.

c. Procedure with brilliant green lactose bile broth:

1) Either:

a) Gently shake or rotate primary fermentation tube showing gas and with a sterile metal loop, 3 mm in diameter, transfer one loopful of medium to a fermentation tube containing brilliant green lactose bile broth, or

b) Gently shake or rotate primary fermentation tube showing gas and insert a sterile wood applicator at least 2.5 cm (1 in.) into the medium. Promptly remove and plunge applicator to bottom of fermentation tube containing brilliant green lactose bile broth. Remove and discard applicator.

2) Incubate the inoculated brilliant green lactose bile broth tube for 48±3 hr at 35±0.5 C.

The formation of gas in any amount in the inverted vial of the brilliant green lactose bile broth fermentation tube at any time within 48±3 hr constitutes a positive Confirmed Test.

3. Completed Test

The Completed Test is used as the next step following the Confirmed Test. It is applied to the brilliant green lactose bile broth fermentation tubes showing gas in the Confirmed Test.

a. Procedure:

1) Streak one or more Endo or eosin methylene blue plates from each tube of brilliant green lactose bile broth showing gas, as soon as possible after the appearance of gas. It is essential that the plates be so streaked as to insure the presence of some discrete colonies, separated by at least 0.5 cm from one another. Careful attention to the following details when streaking plates will result in a high proportion of successful isolations if coliform organisms are present: (*a*) Use an inoculating needle slightly curved at the tip; (*b*) tap and incline the fermentation tube to avoid picking up any membrane or scum on the needle; (*c*) insert the end of the needle into the liquid in the tube to a depth of approximately 5.0 mm; (*d*) streak the plate by bringing only the curved section of the needle in contact with the agar surface so that the latter will not be scratched or torn.

Incubate the plate (inverted, if with glass or plastic cover) at 35±0.5 C for 24±2 hr.

2) The colonies developing on Endo or eosin methylene blue agar may be described as *typical* (nucleated, with or without metallic sheen); *atypical* (opaque, unnucleated, mucoid, pink after 24 hr incubation), or *negative* (all others). From each of these plates fish one or more typical well-isolated coliform colonies or, if no typical colonies are present, fish two or more colonies considered most likely to consist of organisms of the coliform group, transferring each fishing to a lactose broth or a lauryl tryptose broth fermentation tube and to a nutrient agar slant.

The use of a colony counter is recommended to provide optimum magnification when colonies are fished from the plates of selective medium.

If possible, when transferring colonies, take care to choose well-isolated colonies separated by at least 0.5 cm from other colonies and barely to touch the surface of the colony with a flame-sterilized, air-cooled transfer needle, so as to minimize the danger of transferring a mixed culture.

The agar slants and secondary broth tubes are incubated at 35±0.5 C for 24±2 or 48±3 hr if gas is not produced in 24 hr. Gram-stained preparations (see Section 908A.4 below) from those agar slant cultures corresponding to the secondary lactose broth tubes that show gas are examined microscopically.

b. Interpretation: The formation of gas in the secondary lactose broth tube and the demonstration of gram-negative nonspore-forming rod-shaped bacteria in the agar culture may be considered a satisfactory Completed Test, demonstrating the presence of a member of the coliform group in the volume of sample examined.

If, after 48±3 hr, gas is produced in the lactose and no spores or gram-positive rods are found on the slant, the test may be considered completed and the presence of coliform organisms demonstrated.

4. Gram-Stain Technic

The Completed Test for coliform-group organisms requires the determination of gram-stain characteristics of the organisms isolated, as discussed above.

There are various modifications of the Gram stain, many of which have been listed by Hucker and Conn (Section 908E). The following modification by Hucker is valuable for staining smears of pure culture. It is desirable to use a gram-positive and a gram-negative culture as controls for the staining process.

a. Reagents:

1) ***Ammonium oxalate-crystal violet*** (Hucker's)—Dissolve 2 g crystal violet (90% dye content) in 20 ml 95% ethyl alcohol; dissolve 0.8 g of ammonium oxalate monohydrate in 80 ml distilled water; mix the two solutions and age for 24 hr before use; filter through paper into a staining bottle.

2) ***Lugol's solution, Gram's modification***—Grind 1 g iodine crystals and 2 g potassium iodide in a mortar. Add distilled water, a few milliliters at a time, and grind thoroughly after each addition until solution is complete. Rinse the solution into an amber glass bottle with the remaining water (using a total of 300 ml).

3) ***Counterstain***—Dissolve 2.5 g safranin dye in 100 ml of 95% ethyl alcohol. Add 10 ml of the alcoholic solution of safranin to 100 ml of distilled water

4) *Acetone alcohol*—Mix equal volumes of ethyl alcohol, 95%, with acetone.

b. Procedure: Prepare a light emulsion of the bacterial growth on an agar slant in a drop of distilled water on a glass slide. Air-dry or fix by passing the slide through a flame and stain for 1 min with the ammonium oxalate-crystal violet solution. Rinse the slide in tap water; apply Lugol's solution for 1 min.

Rinse the stained slide in tap water. Decolorize with acetone alcohol by holding slide between the fingers and letting acetone alcohol flow across the stained smear until no more stain is removed. Do not over-decolorize. Counterstain with safranin for 15 sec, then rinse with tap water, blot dry with bibulous paper, and examine microscopically.

Cells that decolorize and accept the safranin stain are pink and defined as gram-negative in reaction. Cells that do not decolorize but retain the crystal violet stain are deep blue and are defined as gram-positive.

3.12. Application of Tests to Routine Examinations

The following basic considerations apply to the selection of the Presumptive Test, the Confirmed Test, or the Completed Test in the examination of any given sample of water or wastewater.

1. Presumptive Test

The Presumptive Test without confirmation should not be used routinely; however, it may be applied to the examination of:

a. Any sample of waste, sewage, or water known to be heavily polluted, the fitness of which for drinking water is not under consideration.

b. Any routine sample of raw water in a treatment plant, provided records indicate that the Presumptive Test is not too inclusive for the development of pertinent data.

2. Confirmed Test

The Confirmed Test should be applied as a minimum to all samples.

3. Completed Test

The Completed Test should be applied in the examination of water samples where the results are to be used for the control of the quality of raw or finished waters; or if not applied to all samples, then to such a proportion of them as to establish beyond reasonable doubt the value of the Confirmed Test in determining the sanitary quality of such water supplies. Repeat samples of finished water from the same location that consistently show three or more positive 10-ml portions should be analyzed by the Completed Test.

NOTE: Schematic outlines of the Presumptive, Confirmed, and Completed Tests are shown in Figures 908: 1a and 908: 1b.

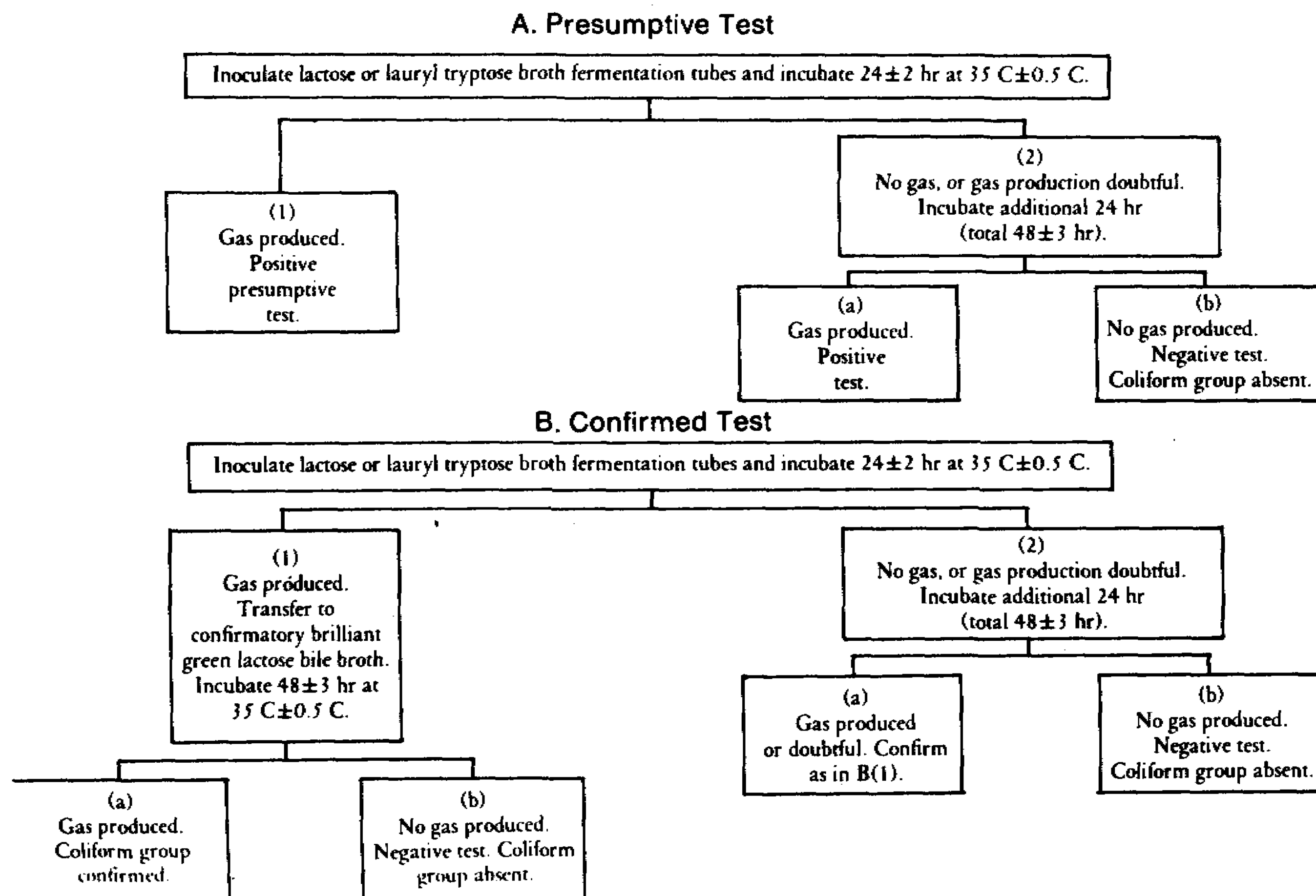

Figure 908:1a. Schematic outline of presumptive and confirmed tests

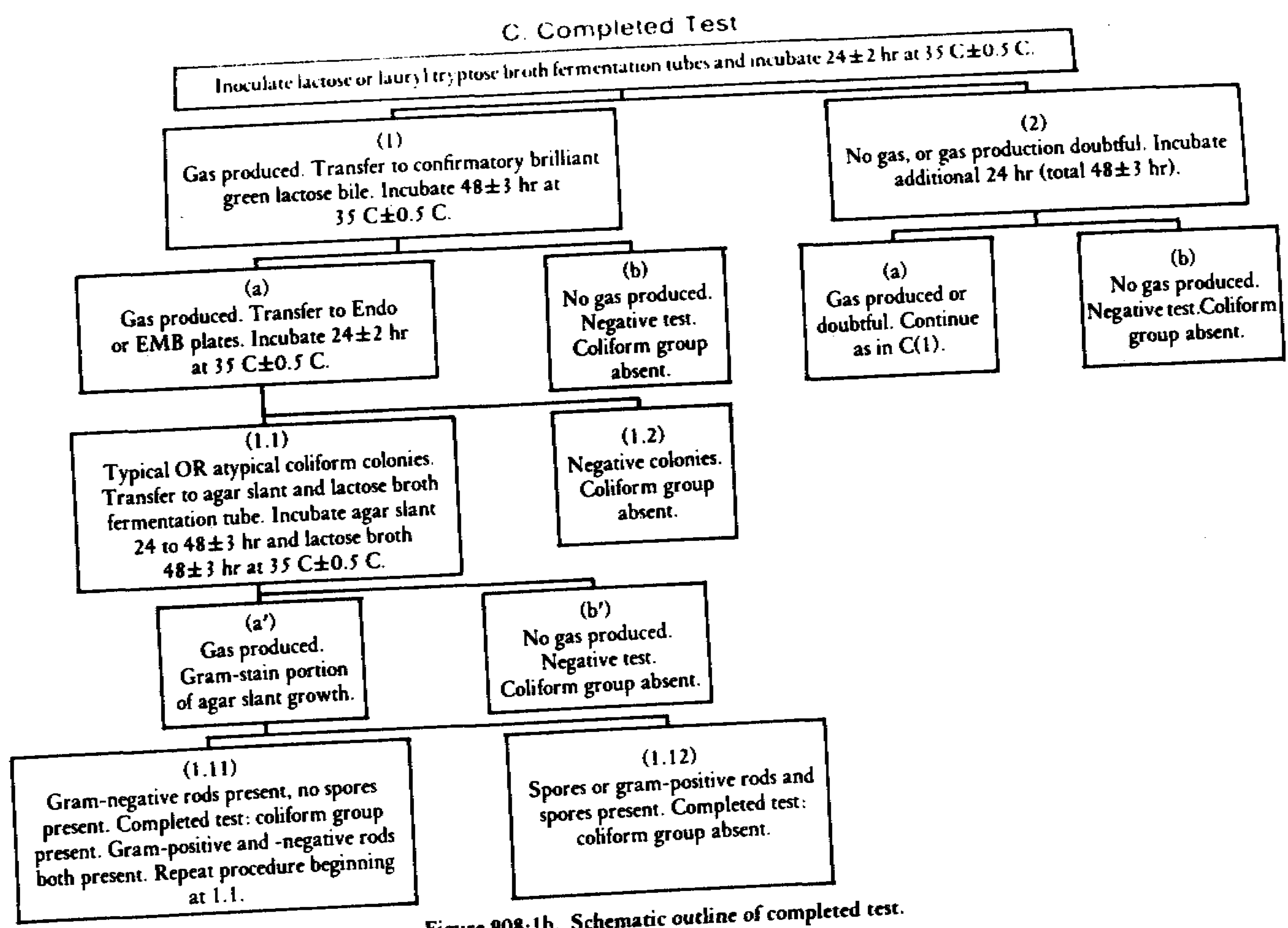

Figure 908:1b. Schematic outline of completed test.

3.13. Fecal Coliform MPN Procedure

Elevated temperature tests for the separation of organisms of the coliform group into those of fecal origin and those derived from nonfecal sources have been used in many parts of the world and with various modifications. Recent modifications in technical procedures, standardization of methods, and detailed studies of members of the coliform group found in the feces of various warm-blooded animals compared with those from other environmental sources have established the value of a fecal coliform determination. This test may be performed either by a multiple-tube procedure as described here or by membrane filter methods as described in the section on the membrane filter technic. The following procedure yields adequate information about the source of the coliform group (fecal or nonfecal) when used as a *confirmatory* test procedure. This multiple-tube procedure cannot be used for direct isolation of coliforms from water but requires prior enrichment in a Presumptive Test medium for optimum recovery of fecal coliforms.

The fecal coliform test is applicable to investigations of stream pollution, raw water sources, sewage treatment systems, bathing waters, seawaters, and general water-quality monitoring. The procedure is not recommended as a substitute for the coliform test in the examination of potable waters, since no coliform bacteria of any kind should be tolerated in a treated water.

1. Fecal Coliform Test (EC Medium)

The fecal coliform test, when executed as described, may be expected to differentiate between coliforms of fecal origin (intestines of warm-blooded animals) and coliforms from other sources. Use EC medium as described in Section 905C, Media Specifications, No. 9, preceding.

a. Procedure: Make transfers from all positive presumptive tubes from the total coliform MPN test to EC medium. This examination may be performed simultaneously with the confirmatory procedure using brilliant green lactose bile broth. Use a sterile metal loop with a minimum 3-mm diam or a sterile wooden applicator to transfer from the positive fermentation tube to EC medium. When making such transfers, first gently shake the presumptive tube or mix by rotating. Inoculated tubes are incubated in a water bath at 44.5±0.2 C for 24±2 hr. Place all EC tubes in the water bath within 30 min after planting. The water depth in the incubator should be sufficient to immerse tubes to the upper level of the medium.

b. Interpretation: Gas production in a fermentation tube within 24 hr or less is considered a positive reaction indicating fecal origin. Failure to produce gas (growth sometimes occurs) constitutes a negative reaction indicating a source other than the intestinal tract of warm-blooded animals. Fecal coliform densities are calculated as described under Estimation of Bacterial Density (Section 908D below).

3.14. Estimation of Bacterial Density

1. Precision of Fermentation Tube Test

It is desirable to bear in mind that unless a large number of portions of sample are examined, the precision of the fermentation tube test is rather low. For example, even when the sample contains 1 coliform organism/ml, about 37% of 1-ml tubes may be expected to yield negative results because of irregular distribution of the bacteria in the sample. When five tubes, each with 1 ml of sample, are used under these conditions, a completely negative result may be expected less than 1% of the time.

Even when five fermentation tubes are used, the precision of the results obtained is not of a high order. Consequently, great caution must be exercised when interpreting, in terms of sanitary significance, the coliform results obtained from the use of a few tubes with each dilution of sample, especially when the number of samples from a given sampling point is limited.

2. Computing and Recording of MPN

The number of positive findings of coliform group organisms (either presumptive, confirmed, or completed) resulting from multiple-portion decimal-dilution plantings should be computed as the combination of positives and recorded in terms of the Most Probable Number (MPN). The MPN, for a variety of planting series and results, is given in Tables 908:I and 908:II. Included in these tables are the 95% confidence limits for each MPN value determined.

The sample volumes indicated in Table 908:II relate more specifically to finished waters. The values may be used in computing the MPN in larger or smaller portion plantings in the following manner: If, instead of portions of 10, 1.0, and 0.1 ml, a combination of portions of 100, 10, and 1 ml is used, the MPN is recorded as 0.1 times the value given in the applicable table.

If, on the other hand, a combination of corresponding portions at 1.0, 0.1,

TABLE 908:I. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 10-ML PORTIONS ARE USED

No. of Tubes Giving Positive Reaction out of 5 of 10 ml Each	MPN Index /100 ml	95% Confidence Limits	
		Lower	Upper
0	<2.2	0	6.0
1	2.2	0.1	12.6
2	5.1	0.5	19.2
3	9.2	1.6	29.4
4	16.	3.3	52.9
5	>16.	8.0	Infinite

TABLE 908:II. MPN INDEX AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN VARIOUS NUMBERS OF TUBES ARE USED PER DILUTION (10 ML, 1.0 ML, 0.1 ML)

Combination of Positives	Tubes per Dilution					
	3			5		
	MPN Index /100 ml	95% Confidence Limits		MPN Index /100 ml	95% Confidence Limits	
		Lower	Upper		Lower	Upper
0-0-0	<3			<2		
0-0-1	3	<0.5	9	2	<0.5	7
0-1-0	3	<0.5	13	2	<0.5	7
0-2-0	—			4	<0.5	11
1-0-0	4	<0.5	20	2	<0.5	7
1-0-1	7	1	21	4	<0.5	11
1-1-0	7	1	23	4	<0.5	11
1-1-1	11	3	36	6	<0.5	15
1-2-0	11	3	36	6	<0.5	15
2-0-0	9	1	36	5	<0.5	13
2-0-1	14	3	37	7	1	17
2-1-0	15	3	44	7	1	17
2-1-1	20	7	89	9	2	21
2-2-0	21	4	47	9	2	21
2-2-1	28	10	150	—		
2-3-0	—			12	3	28
3-0-0	23	4	120	8	1	19
3-0-1	39	7	130	11	2	25
3-0-2	64	15	380	—		
3-1-0	43	7	210	11	2	25
3-1-1	75	14	230	14	4	34
3-1-2	120	30	380	—		
3-2-0	93	15	380	14	4	34
3-2-1	150	30	440	17	5	46
3-2-2	210	35	470	—		
3-3-0	240	36	1,300	—		
3-3-1	460	71	2,400	—		
3-3-2	1,100	150	4,800	—		
3-3-3	≥2,400			—		
4-0-0	—			13	3	31
4-0-1	—			17	5	46
4-1-0	—			17	5	46
4-1-1	—			21	7	63
4-1-2	—			26	9	78
4-2-0	—			22	7	67
4-2-1	—			26	9	78
4-3-0	—			27	9	80
4-3-1	—			33	11	93
4-4-0	—			34	12	93

TABLE 908:II. *(Continued)*

Combination of Positives	Tubes per Dilution					
	3			5		
	MPN Index /100 ml	95% Confidence Limits		MPN Index /100 ml	95% Confidence Limits	
		Lower	Upper		Lower	Upper
5-0-0	—			23	7	70
5-0-1	—			31	11	89
5-0-2	—			43	15	110
5-1-0	—			33	11	93
5-1-1	—			46	16	120
5-1-2	—			63	21	150
5-2-0	—			49	17	130
5-2-1	—			70	23	170
5-2-2	—			94	28	220
5-3-0	—			79	25	190
5-3-1	—			110	31	250
5-3-2	—			140	37	340
5-3-3	—			180	44	500
5-4-0	—			130	35	300
5-4-1	—			170	43	490
5-4-2	—			220	57	700
5-4-3	—			280	90	850
5-4-4	—			350	120	1,000
5-5-0	—			240	68	750
5-5-1	—			350	120	1,000
5-5-2	—			540	180	1,400
5-5-3	—			920	300	3,200
5-5-4	—			1,600	640	5,800
5-5-5	—			≥2,400		

and 0.01 ml is planted, record 10 times the value shown in the table; if a combination of portions of 0.1, 0.01, and 0.001 ml is planted, record 100 times the value shown in the table; and so on for other combinations.

When more than three dilutions are used in a decimal series of dilutions, the results from only three of these are used in computing the MPN. To select the three dilutions to be used in determining the MPN index, taking the system of five tubes of each dilution as an example, the highest dilution that gives positive results in all five portions tested (no lower dilution giving any negative results) and the two next succeeding higher dilutions should be chosen. The results at these three volumes should then be used in computing the MPN index. In the examples given below, the significant dilution results are shown in

boldface. The number in the numerator represents positive tubes; that in the denominator, the total tubes planted; the combination of positives simply represents the total number of positive tubes per dilution:

Example	1 ml	0.1 ml	0.01 ml	0.001 ml	Combination of positives
(*a*)	5/5	5/5	2/5	0/5	5-2-0
(*b*)	5/5	4/5	2/5	0/5	5-4-2
(*c*)	0/5	1/5	0/5	0/5	0-1-0

In *c*, the first three dilutions should be taken, so as to throw the positive result in the middle dilution.

When a case such as shown below in line *d* arises, where a positive occurs in a dilution higher than the three chosen according to the rule, it should be incorporated in the result for the highest chosen dilution, as in *e*:

Example	1 ml	0.1 ml	0.01 ml	0.001 ml	Combination of positives
(*d*)	5/5	3/5	1/5	1/5	5-3-2
(*e*)	5/5	3/5	2/5	0/5	5-3-2

When it is desired to summarize with a single MPN value the results from a series of samples, the geometric mean, the arithmetic mean, or the median may be used.

Table 908:II does not include all positive combinations; however, the most likely ones are shown. If unlikely combinations occur with a frequency greater than 1% it is an indication that the technic is faulty or that the statistical assumptions underlying the MPN estimate are not being fulfilled. The MPN for combinations not appearing in the table, or for other combinations of tubes or dilutions, may be estimated by Thomas' simple formula:

$$\text{MPN/100 ml} = \frac{\text{no. of positive tubes} \times 100}{\sqrt{\left(\begin{array}{c}\text{ml sample in}\\ \text{negative tubes}\end{array}\right) \times \left(\begin{array}{c}\text{ml sample in}\\ \text{all tubes}\end{array}\right)}}$$

While the MPN tables and calculations are described for use in the coliform test, they are equally applicable to determination of the MPN of any organisms provided a suitable test is available.

908 E. Bibliography

Standard Tests

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4.EXPERIMENTAL DIGESTERS

4.1.Definition and problems encounterred in anaerobic reactors.

Unadapted seed material: Reactor start-up is defined as the first start-up of a reactor seeded with anadapted seed material.The first start-up is mea meant,as opposed to the start-up after a standstill.The latter is referred to as restart.Unadapted seed material in this respect is considered to be anaerobic sludge with the characteristics mentioned in Table 4.1.

TABLE 4.1.Characteristics of unadapted seed sludge for UASB reactor start-up.

1.The seed material is obtained from an anaerobic treatment system without biomass retention,such as conventional mixed digesters,ruminants,septic tank,or fresh water sediments.

2.The sludge exerts a specific methanogenic activity of less than 0,2 Kg CH_4-COD/Kg VSS.day.

3.The settleability of the organic fraction of the sludge is poor.

4.The bacterial population of the sludge is unbalanced with respect to the composition needed for the simultaneous degradation of all waste ingredients

5.The sludge is not adapted to specific inhibitors in the wastewater (such as sulphide,ammonia,etc.).

Start-up time :A practical definition of UASB reactor start-up time would be the time required to meet the desigh criteria of the system.For research purposes obviously another definition is required.
A more scientific definition of the start-up period is the time after which the sludge characteristics do not chage any more,when treating a wastewater of a constant composition under fixed conditions.

Duration : Because of the much lower growth rates of anaerobic bacteria as compared to aerobic bacteria,the first start-up of an anaerobic treatment plant always takes more time,than that of an aerobic one.
The slow growth rate of anaerobes also has a magnifying effect upon disturbing factors.If an inhibiting compound in the waste diminishes the net

growth,the start-up time will increase accordingly,e.g.from 70 to at least 140 days in case the growth rate is decreased by 50%.In an aerobic treatment plant a comparable increase would be in the order of days or week instead of months.

Specific activity of sludge : The specific activity of anaerobic sludge expressed as g CH_4-COD/g VSS.day is an important parameter in several respects.

When start-up a reactor the specific activity of the seed sludge together with the amount of sludge present determines the maximum initial organic loading rate.

It provides information about the development of the sludge from a digested sewage sludge during subsequent stages of an UASB-reactor start-up.

In order to calculate the specific activity of sludge the methane produc - tion rate or COD removal rate and the amount of sludge present in the re - actor,as well as the fraction of the sludge activity participating in the digestion process must be known.Specific activity of sluge is calculated b by equation :

$$\text{Activity} = \frac{\text{Max. slope}}{\text{absolute amount of sludge-VSS}} \quad (\text{g } CH_4\text{-COD/g VSS.day})$$

The Fig.4.1 shows a example of this calculation

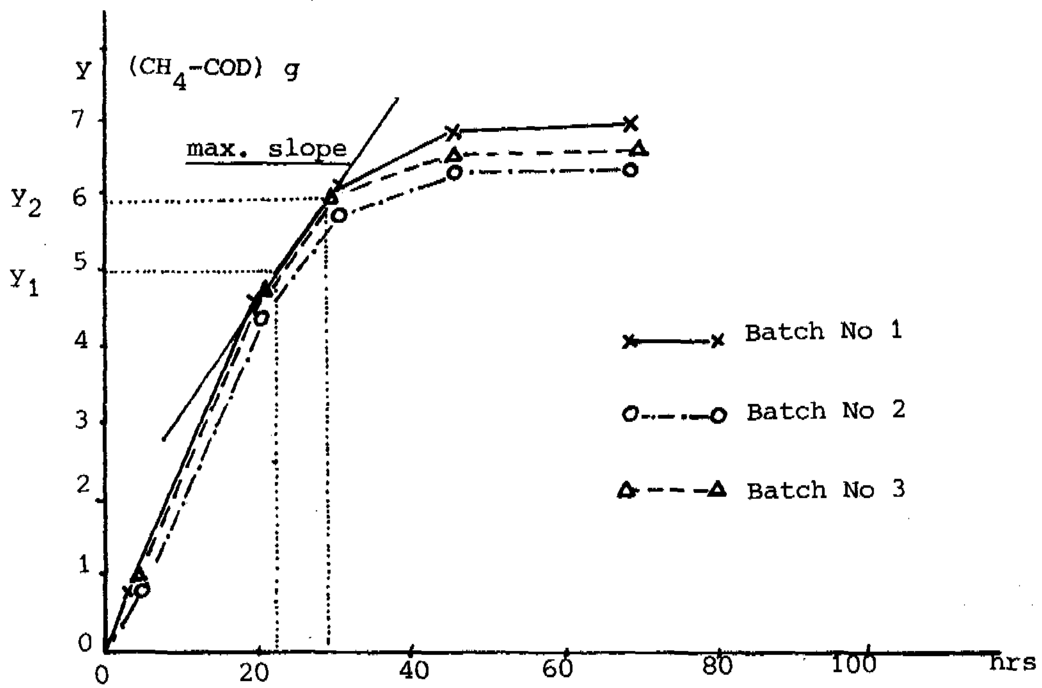

FIGURE 4.1. Total CH_4-COD in 3 batches of 4th feeding.

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$$\text{max. slope} = \frac{y_2 - y_1}{t_2 - t_1} = \frac{5,9 - 5,0}{30 - 23} = 0,1285 \text{ g COD/h}$$

$$\text{Activity} = \frac{0,1285}{12,24} = 0,0104 \text{ g COD/g VSS.h}$$

$$\text{or : } 0,0104 \times 24 = 0,2496 \text{ g COD/g VSS.day}$$

Nature of the seed : Theorically any medium containing the proper bacterial flora can be used as seed sludge for an UASB reactor.Examples of possible seed materials are (digested) manure,fresh water sediments,septic tank sludge ,(digested) sewage sludge and surplus sludge from anaerobic treatment plants.

Lag phases : The response of an adapted seed sludge to a new waste may include a lag phase.Among the factors influencing the occurrence and the lenth of a lag phase are the concentration of active biomass,the mixing intensity and the initial substrate concentration.
These factors were examined in batch fed stirred tank digesters with digested sewage sludge.

Growth yield : The magnitude of the growth yield influences the lenth of the start-up period.The growth yield,i.e.the amount of new sludge formed per unit of COD converted,depens on the nature of the substrate,as well as on environmental factos.

Sludge wash-out and sludge retention : During the start-up of an UASB reactor the upward velocity of both water and gas causes finaly dispersed and poorly settling sludge particles to wash out from the reactor.This wash-out is not necessarily a problem,as sludge wash-out during start-up represents a key feature of the UASB concept,i.e.the selection pressure exerted upon the sludge particles.This selection utimately leads to the development of a high highly settleable,granular sludge.

4.2. Experimental equipments

Fig.4.2 and 4.3 show the batch-reactor and UASB reactor for anaerobic treatment research. Batches-digesters are stirred intermittlently 1 sec every

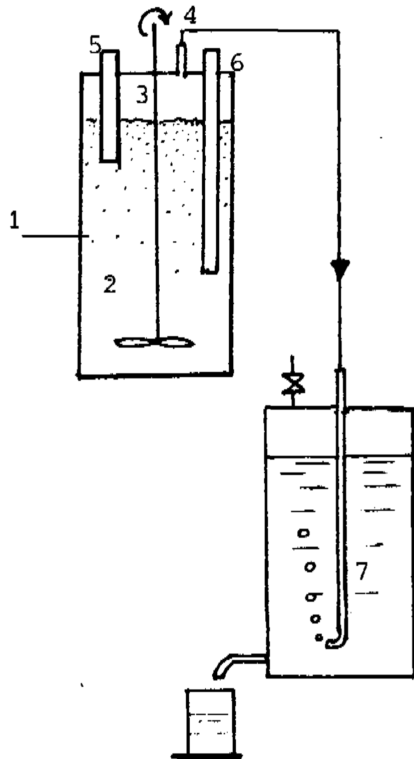


FIGURE 4.2 Batch reactor used for granular sludge activity tests.

1. batch reactor,
2. sludge mixed liquor,
3. headspace,
4. gas outlet,
5. inlet point for pH electrode,
6. sampling port,
7. liquid displacement system for gas measurement.

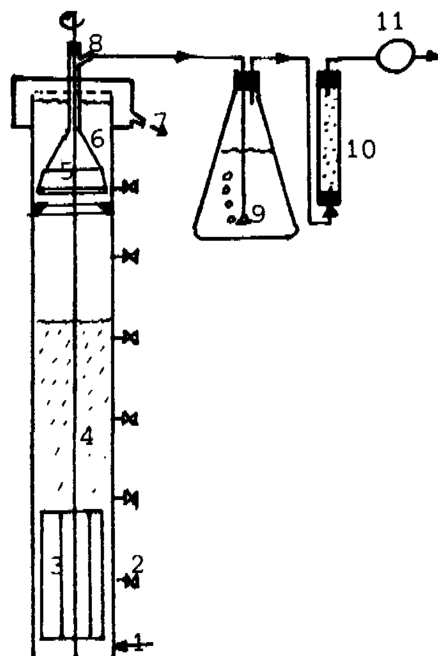


Figure 4.3 Laboratory UASB reactor

1. influent inlet points,
2. sampling ports,
3. flat blade impeller,
4. sludge bed,
5. gas collector,
6. settler,
7. effluent discharge,
8. gas outlet,
9. CO_2 and H_2S stripping device,
10. granular soda lime,
11. gas meter.

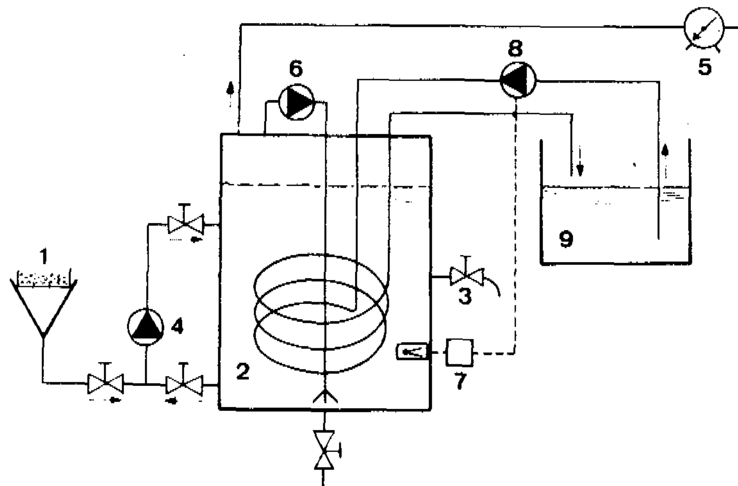


Fig.4.4.Small pilot plant experiments

1.inlet,
2.digestion unit,
3.outlet pipe,
4.sludge recirculation ,
5.gas meter,
6. gas reciculation pump,
7.thermo couple and
thermostatic control,
8.water cijculation pump,
9.hot water tank (about 50°C).

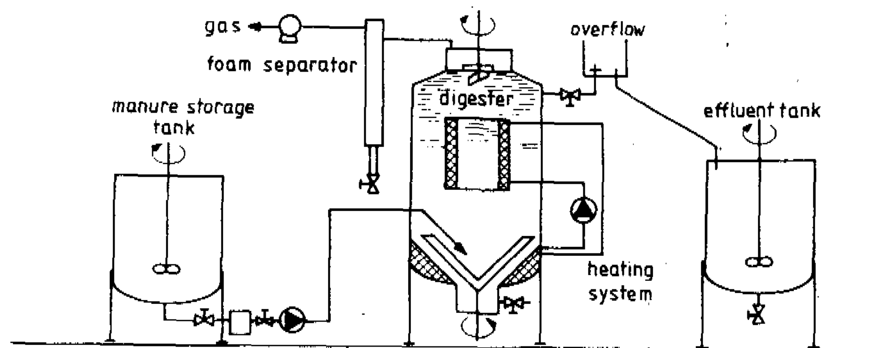


Fig.4.5. Experimental pilot plant digester (6 m^3).

(From A.F.M.van Velsen : Anaerobic digestion of piggery waste.Depart.of Water Pollution Control Wageningen Agricultural University,the Netherlands)

min a wet volume 1,5;2,5;..6 liters.The reactors are connected to a flask of Mariotte for gas production measurement.CO_2 is removed from the gas st stream by using 1% alkaline solution.

The UASB reactors are equiped with a stirring assembly,which is used only at gas production rates smaller than $1m^3/m^3$.d (intermittently :30 sec at 10 - 20 rpm each 10-20 min).

4.3. Examples of Batche-experiments.

Batch experiments of granular sludge:

Fig.4.2 shows the batch-reactor.A mean sludge addition of 5 Kg VSS.m^{-3} is supplid to the batch.The seed sludge is diluted with warm deoxygenated tap water.

The substrate used is mixture of acetic-C_2 , propionic-C_3 , butyric-C_4 acid.The concentration of each invidual volatile fatty acid (VFA) amounted 0,6 Kg.m^{-3}.This is equivalent to a total COD of 6,58 : 2,5 = 2,632 Kg COD m^{-3}.The amount of the VFA´solution is added to 2,5 l batch amounted 15 ml each feeding.The VFA are supplied parly as their sodium salts $NaHCO_3$ in order to obtain a pH 7-7,2.

Per 2,5 liters of mixed liquor the following nutrients are added :5 ml N+P solution (from 174 g NH_4Cl/l and 28,3 g/l KH_2PO_4), 5 g $NaHCO_3$, 5 ml Na_2-SO_4 as well as trace element.Zehnder trace element solution composition is geven in Table 4.2.

All experiments are conducted in a temperature controlled room (at 30^oC).

TABLE 4.2. Compounds of the trace element-solution.

Compound	mg/l	Compound	mg/l
H_3BO_3	50	$FeCl_2.4H_2O$	2000
$ZnCl_2$	50	$MnCl_2.4H_2O$	500
$CuCl_2.2H_2O$	30	$(NH_3)_6Mo_7O_{24}.4H_2O$	50
$AlCl_3.6H_2O$	90	$CoCl_2.6H_2O$	2000
$NiCl_2.6H_2O$	50	$Na_2SeO_3.5H_2O$	100
EDTA	1000	HCl 36 %	1 ml
Resazurin Standard Fluka= $C_{12}H_6NNaO_4$ (pH = 3,8-6,5)			200

Fig. 4.4;4.5. show the results of the total CH_4-COD g;VFA-concen - tration;CH_4-production.

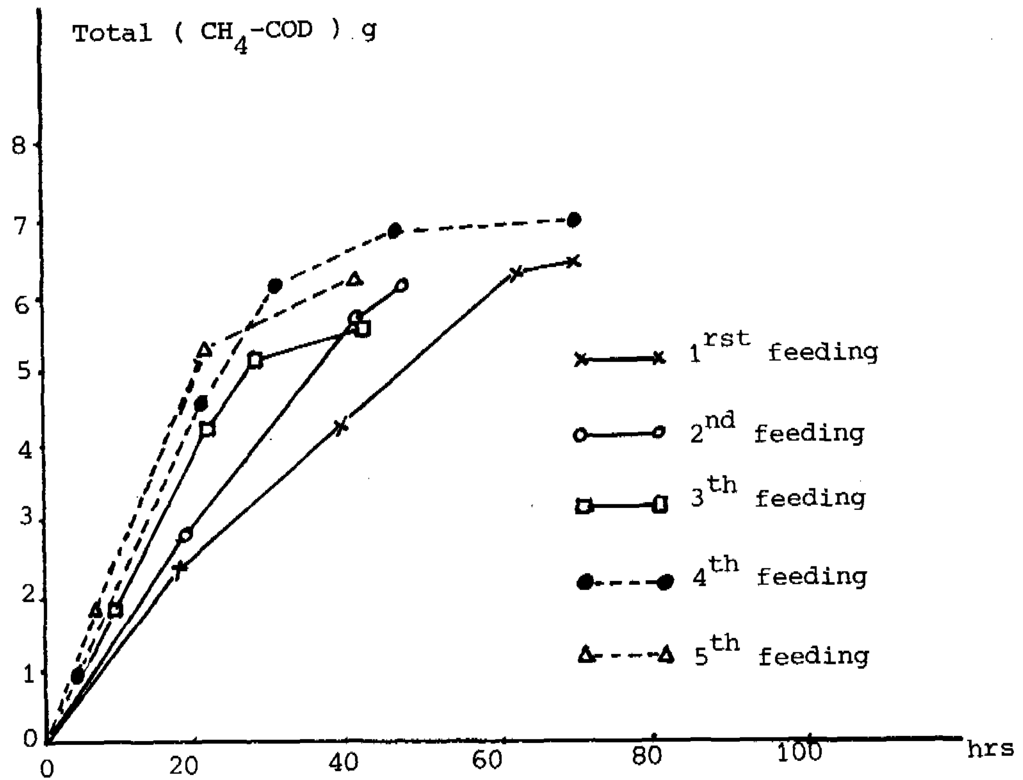


FIGURE 4.4.. Total CH_4-COD in Batch No 1

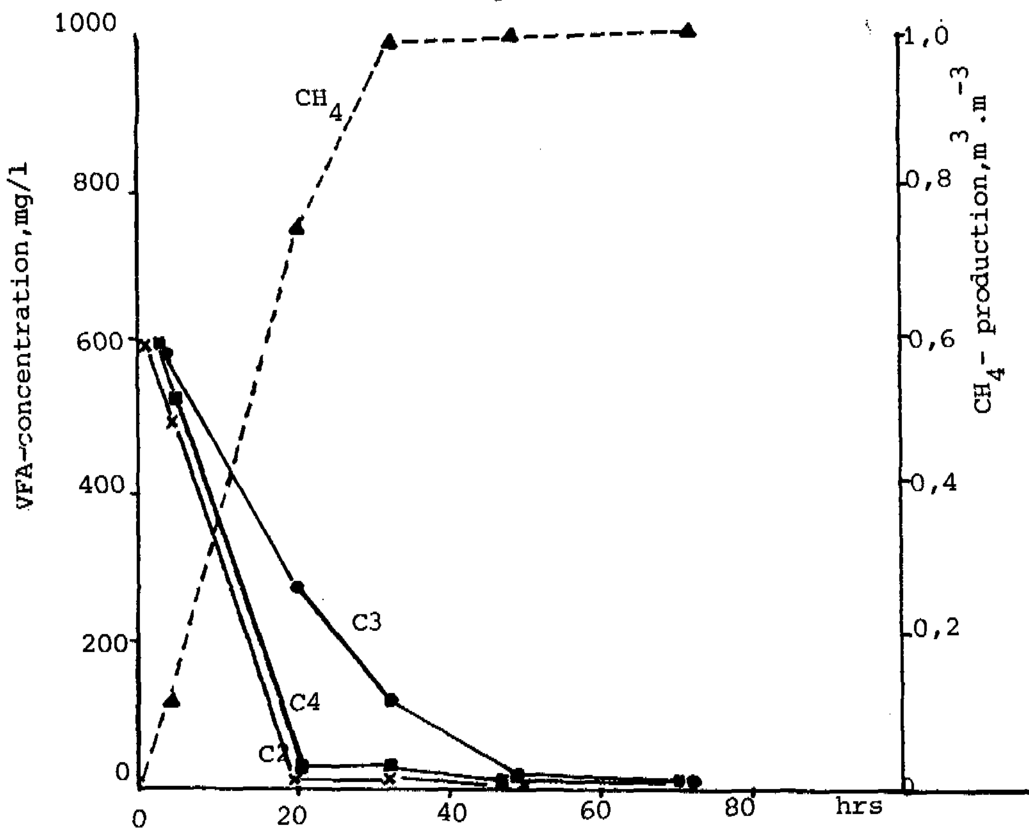
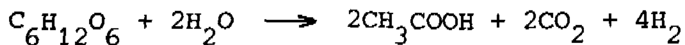


FIGURE 4.5.. The VFA-concentration and CH_4-production of 4th Feeding (batch No 1).

Batch experiments with Vinasse :

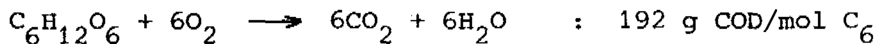
Because the vinasse contens a lot of yeast we have to remove the yeast and as a result we have the filtrated vinasse.We start the experiments with the filtrated vinasse.

Hydrolysis reaction of sugar :

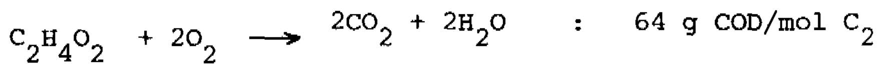


$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

Amount of COD necessary for complete oxidation of sugar :



$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \quad : \quad 192 \text{ g COD/mol } C_6$$



$$C_2H_4O_2 + 2O_2 \longrightarrow 2CO_2 + 2H_2O \quad : \quad 64 \text{ g COD/mol } C_2$$

$$= 128 \text{ g COD/mol } C_6$$

$$H_2 + \tfrac{1}{2} O_2 \longrightarrow H_2O \quad : \quad 16 \text{ g COD/mol } H_2$$

$$= 64 \text{ g COD/mol } C_6$$

So :

$$C_6H_{12}O_6 \text{ — } 100\ \% \quad = 192 \text{ g COD/mol } C_6$$

$$CH_3COOH = \frac{128}{192} \times 100 \quad = 67\ \% \text{ COD}$$

$$H_2 = \frac{64}{192} \times 100 \quad = 33\ \% \text{ COD}$$

About H_2 and SO_4^{-2} :

$$4H_2 + SO_4^{-2} \longrightarrow S^{-2} + 4H_2O$$

Amount of COD necessary for reduction of SO_4^{-2} :

$$S^{-2} + 2O_2 \longrightarrow SO_4^{-2} \quad : \quad 64 \text{ g COD/mol } SO_4 \text{ ,or}$$

$$\frac{64}{96} = 0{,}67 \text{ g COD/g } SO_4$$

Vinasse influent-concentration is 85 g COD/l with COD:SO_4 ratio:10:1 g/g so 8,5 g SO_4/l complete reduction requires :

$8{,}5 \times 0{,}67 = 5{,}7$ g COD/l

$\frac{5{,}7}{85} \times 100\ \% = 6{,}7\ \%$ of influent COD

Influent 85 g COD/l - anaerobic digestion :

— 49,55 g/l (58,3%) CH_4-COD

— 5,7 g/l (6,7 %) H_2S-COD

— 29,75 g/l (35 %) non-biodegradable COD

Three batches (2,5 l) are used for determination of the maximum activity CSM granular sludge with different concentrations vinasse: 5 (batch No 1), 10 (batch No 2), and 20 g COD/l (batch No 3).
After settling, the supernatant is removed from the batch reactor, while the headspace is flushed with N_2-gas. The batches are filled with the desired amount of concentrated vinasse plus tapwater (deoxigenated).
Influent COD is 84,5 g/l
Influent SO_4 is 6,4 g/l

For 5 g COD/l : Amount of vinasse (ml)(batch No 1):
5 : 84,5 = 0,0529 = 59,2 ml/l
and for 2,5 l batch : 59,2 x 2,5 = 148 ml undiluted vinasse

For 10 g COD/l : (batch No 2) :

(10 : 84,5) x 2,5 x 1000 = 295,86 ml

For 20 g COD/l : (batch No 3) :

(20 : 84,5) x 2,5 x 1000 = 591,7 ml

Before feeding, the vinasse volume : 10 liters is neutralized to pH = 7,0 and is added 63,1 g $Na_2SO_4.10H_2O$, 6,25 g KH_2PO_4 and 20 ml trace elements.
In order to provide buffer capacity $NaHCO_3$ is added into batches 0,84 g $NaHCO_3$/g COD for vinasse concentrations of 5 and 10 g COD/l, 0,42 g $NaHCO_3$/ g COD for vinasse concentration of 20 g COD/l.
The reactors are equiped with stirrings with pause 1 min, and puls 1 min.

Analysis:

The following analysis are performed for this study :

CH_4-production	daily,
pH	daily,
VFA-concentration	daily,
SO_4^{-2}, H_2S (for batch No 1)	daily,
(for batch No 2, No 3)	once every one/two days.

During experiment when pH $<$6,5 add $NaHCO_3$ in order to neutralize to pH about 7,0.
After the maximum CH_4-production had been achieved we determine COD with sulphide has been removed by air stripping.

Fig.4.6 shows the total CH_4-COD and SO_4^{-2} concentrations and Fig.4.7 shows the accumulation of VFA in batch-reactors with different COD concentrations.

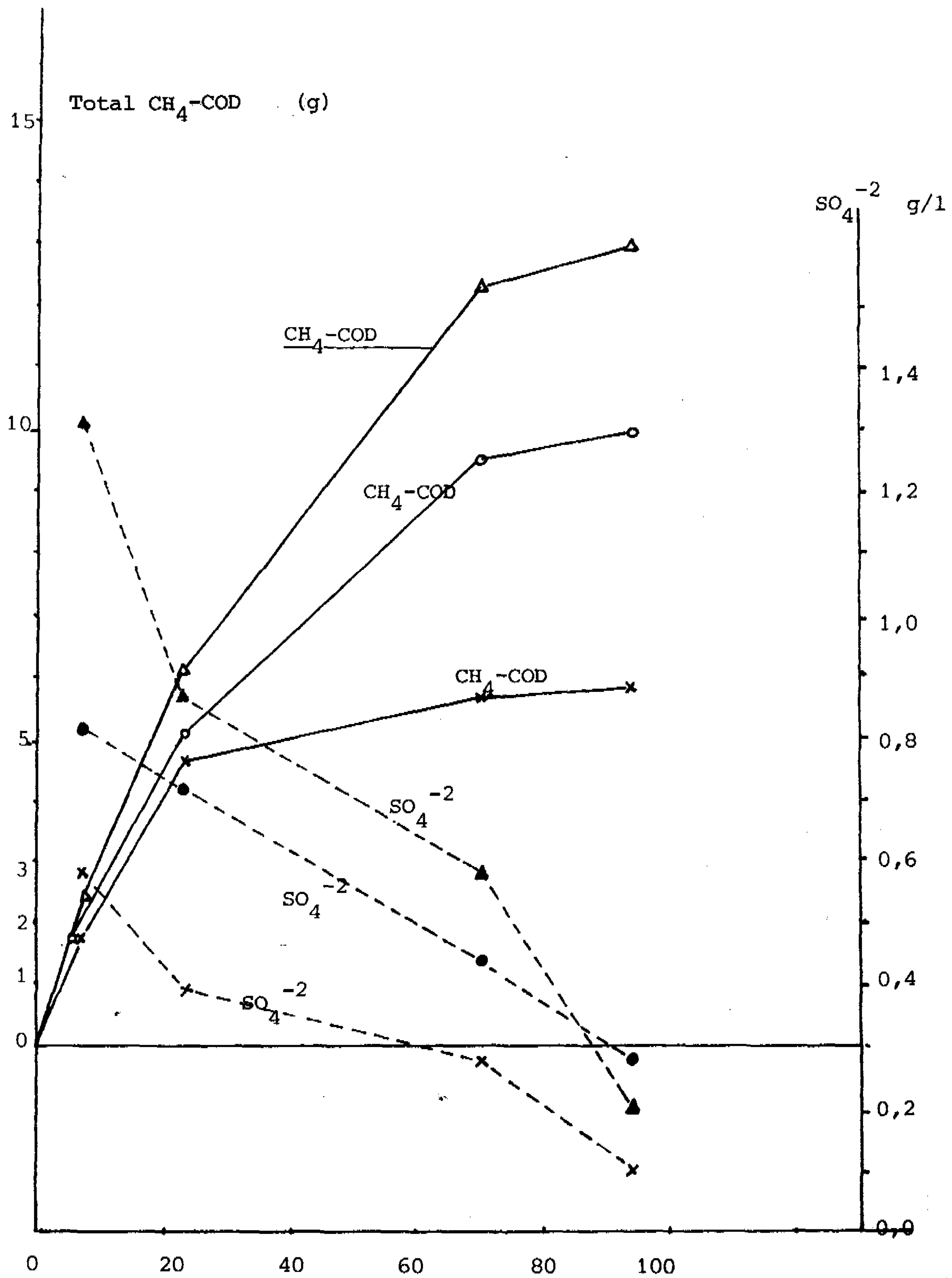


FIGURE 4.6. Total CH_4-COD and SO_4^{-2} concentrations.

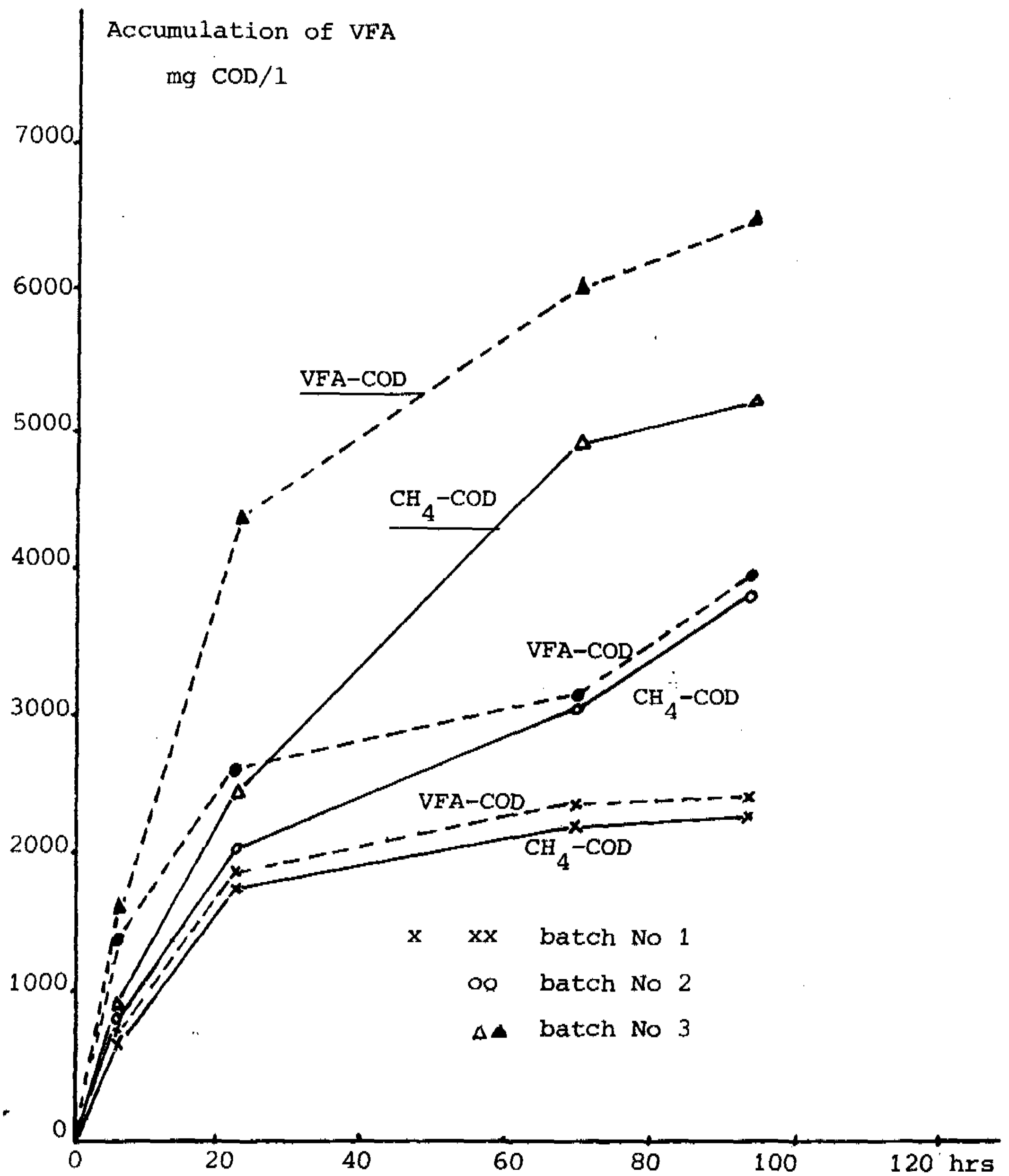


FIGURE 4.7. Accumulation of VFA in batch-reactors

4.4. Example of some calculations

Calculation of COD from methane production:

Calculation of COD from methane production at $30^{o}C$, 1 atm :

Molar volume :

$$\frac{273 + 39}{273} \times 22,4 = 24,84 \text{ l/mol}$$

where 22,4 is molar volume of gas at $0^{o}C$, 1 atm.

$$CH_4 + 2O_2 \longrightarrow CO_2 + 2H_2O$$

$$a \text{ ml } CH_4 = \frac{a}{24,86} \times 64 \text{ mg COD} = a \ . \ 2,574 \text{ mg COD}$$

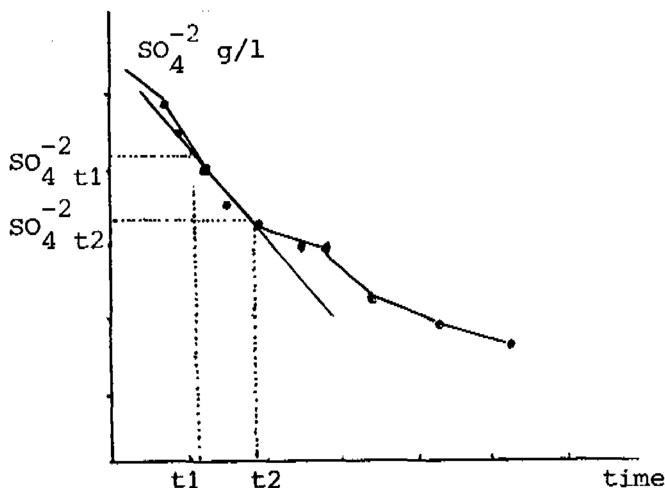
In our experiments we use Mariotte-flask for collection of gas, so that:

$$a \text{ ml } CH_4 = (0,96 \times 2,574) \ a \text{ mg COD} = 2,471 \ a \text{ mg COD}$$

Calculation of the sulphate reduction activity:

The sulphate reduction activity is calculated from the maximum slope of the sulphate concentration curves (see Figure 4.8).

$$\text{Sulphate red. act.} = \frac{\dfrac{\left\{ \left[SO_4^{-2}\right]_{t1} - \left[SO_4^{-2}\right]_{t2} \right\} \times 0,67}{t2 - t1}}{\text{sludge concentration (g VSS/l)}} \quad \text{(g COD/g VSS.h)}$$



where 0,67- amount of COD necessary for reduction of SO_4^{-2}, g COD/g SO_4,

Sludge concentration = $\frac{88,68 \times 140}{1000 \times 2,5}$ (g VSS/l)

SO_4^{-2}-sulphate conc. g/l;

t_1, t_2 - time

FIGURE 4.8. Sulphate conc.

Calculation of the COD conversion:

Calculation of the COD conversion normalized to $COD_{t=o}$:

The COD conversion normalized to $COD_{t=o}$ is calculated by equation:

$$Y = \frac{a \times 2,471}{COD_{t=o} \times 2,5}$$

where a $-CH_4$-production, ml,

2,471- ml gas collected in Mariotte-flask at 30^oC and at 1 atm, mg COD,

$COD_{t=o}$ -COD concentration at t = 0,

2,5 - volume of batch.

The COD conversion normalized to amount of sludge is calculated by equation :

$$Y' = \frac{a \times 2,471}{X \text{ g VSS}}$$

where X- absolute amount of sludge = 12,42 g VSS/l.

Fig.4.9;4.10 show the COD conversion normalized to $COD_{t=o}$ and to the amount of sludge.

Calculation of stock solution containing sucrose and VFA-COD

Stock solution is prepared for the study of granulation of anaerobic sludge in UASB reactors containing sucrose-substrates.

Stock solution for blank reactor A contained 95 % sucrose and 5 % VFA-COD. COD influent is 3000 mg/l. The amount of acetic acid, propionic acid, and sucrose are shown in Table 4.3.

TABLE 4.3 Composition of the subtrates for the blank reactor A

Compounds	COD,mg/l	ml added/l	gr. added/l
CH_3COOH	70	0,063	-
CH_3CH_2COOH	80	0,053	-
Sucrose	2850	-	2,672
Total :	3000 mg / l		

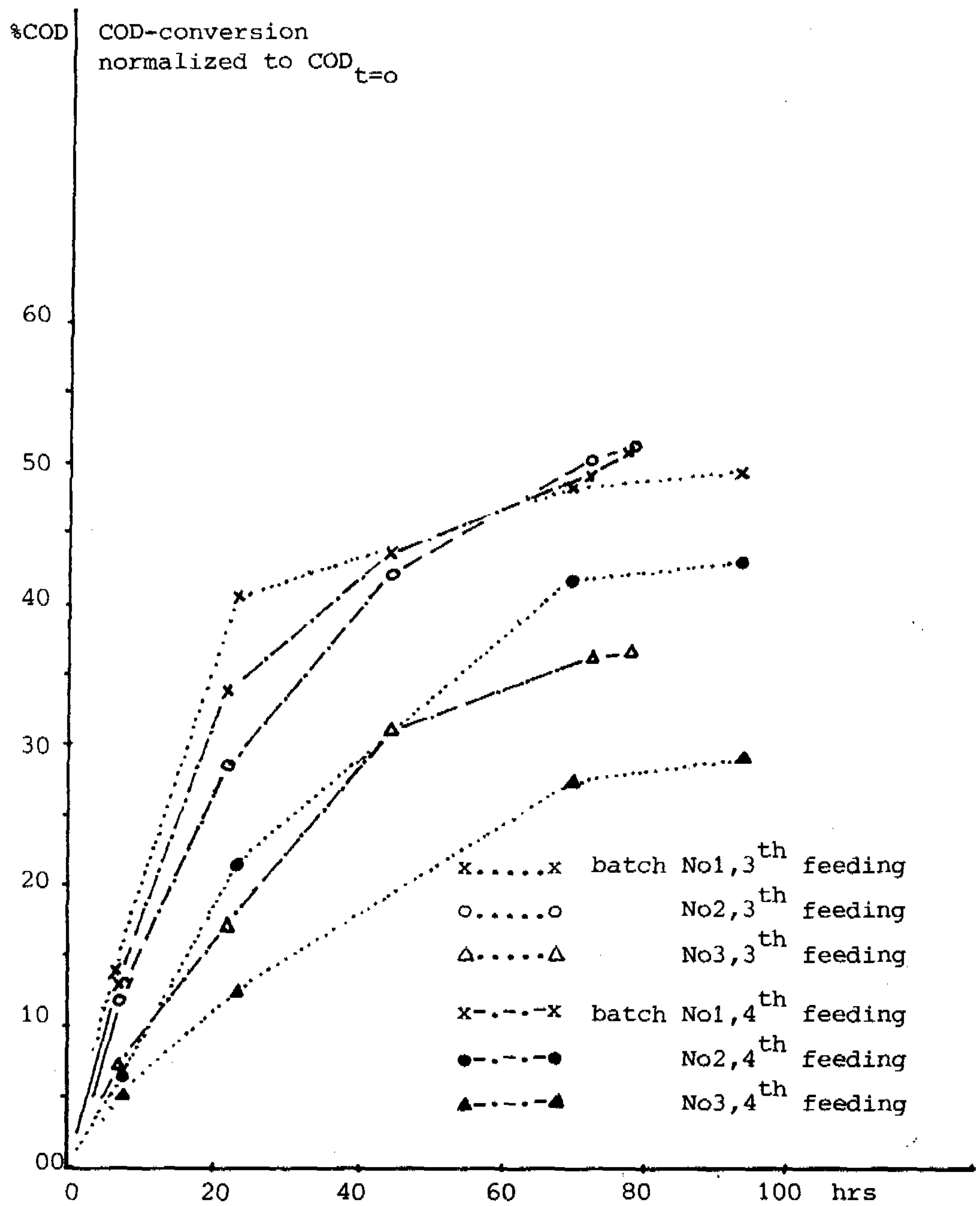


FIGURE 4.9. COD conversion to $COD_{t=o}$ in batch-reactors with vinasse with different COD concentrations of 3th and 4th feedings

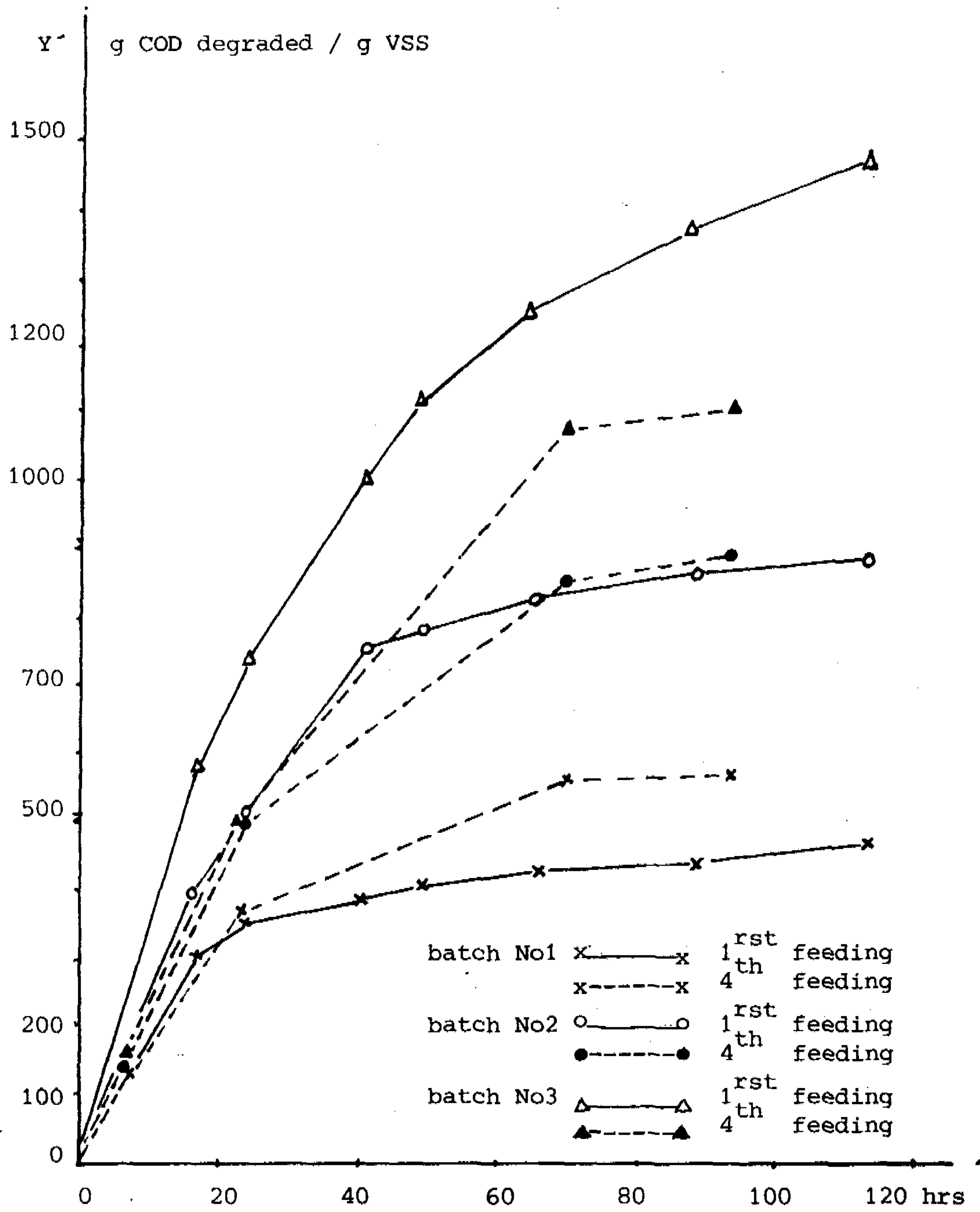


FIGURE 4.10. COD-conversion normalized to amount of sludge of 1rst and 4th feedings.

The COD:N:P:S ratio is kept between 300:5:1:1 by addition of appropriate amount of NH_4Cl (189,3 mg/l), KH_2PO_4 (43,87 mg/l), and Na_2SO_4 (44,38 mg/l) 20 meq $NaHCO_3$/g COD is added for buffering and 0,4 ml/l of trace ele - ment solution is added to the feed. This preparation is based on assumed growth yield of 20%.

Stock solution for the reactor B contained 95% sucrose, 5% VFA-COD, and Na_2SO_4 with the COD:SO_4 ratio 5:1 by addition of appropriate amount of Na_2SO_4 : 976,00 mg/l. 20 meq $NaHCO_3$/g COD is added, and 0,4 ml/l of a trace element solution is added to the feed.

Seed sludges

In our experiment we use digested sewage sludge. In this study, initial sludge loading is : 0,10 gCOD/g VSS.day, therefore the volumetric loading rate :

$$0,1 \times \frac{300}{23,5} = 1,276 = 1,3 \text{ Kg COD } / m^3.\text{day}$$

and the amount of seed sludge added to the reactors was :

$$\frac{1,3}{0,1} \times 23,5 = 305,5 \text{ g VSS}$$

The COD influent concentration is 3 g/l COD so each day :

305,5 x 0,1 = 30,55 g COD / day applied

and the initial flow to the reactors amounts are :

30,55 : 3 = 10,18 l/day

Amount of stock solution :

$$\frac{1,3 \times 23,5}{30,55} = 1 \text{ l/day}$$

and amount of tapwater is : 10,18 - 1 = 9,18 l /day

The laboratory UASB reactors A and B used in this study is geven in Figure 4. 3.

The reactors are equiped with a stirring assembly with the time inter - mittenly : 30 sec at 10-20 rpm each 30 min.

The results of the starting up experiment with sucrose-containing synthetic wastewater are shown in Fig.4.11 and 4.12 (for example, the loading rate (V_l) and gas-production rate (V_g) during the starting up period).

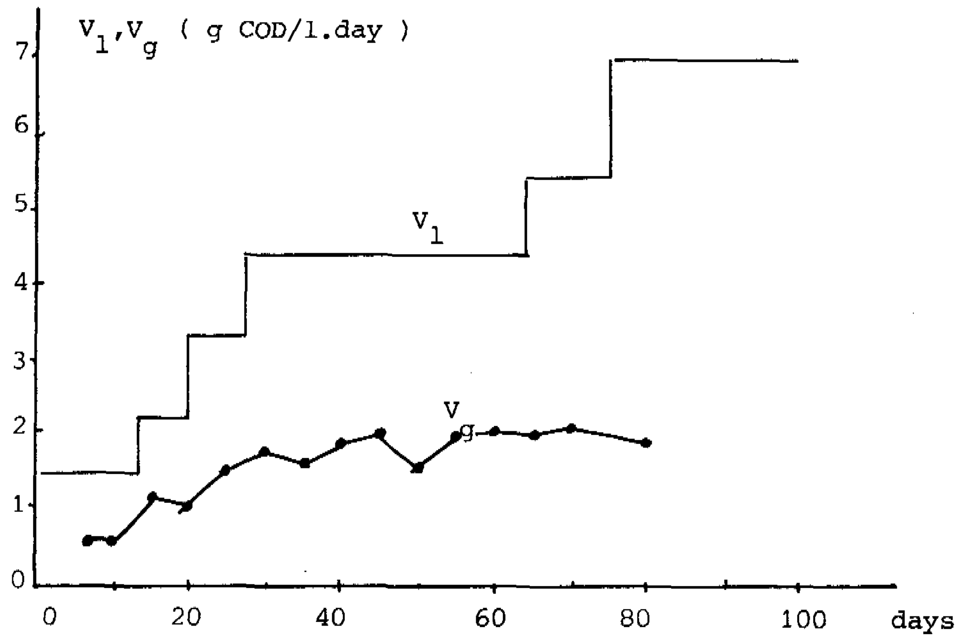


Fig.4.11. Loading rate (V_l) and gas-production rate (V_g) Reactor B

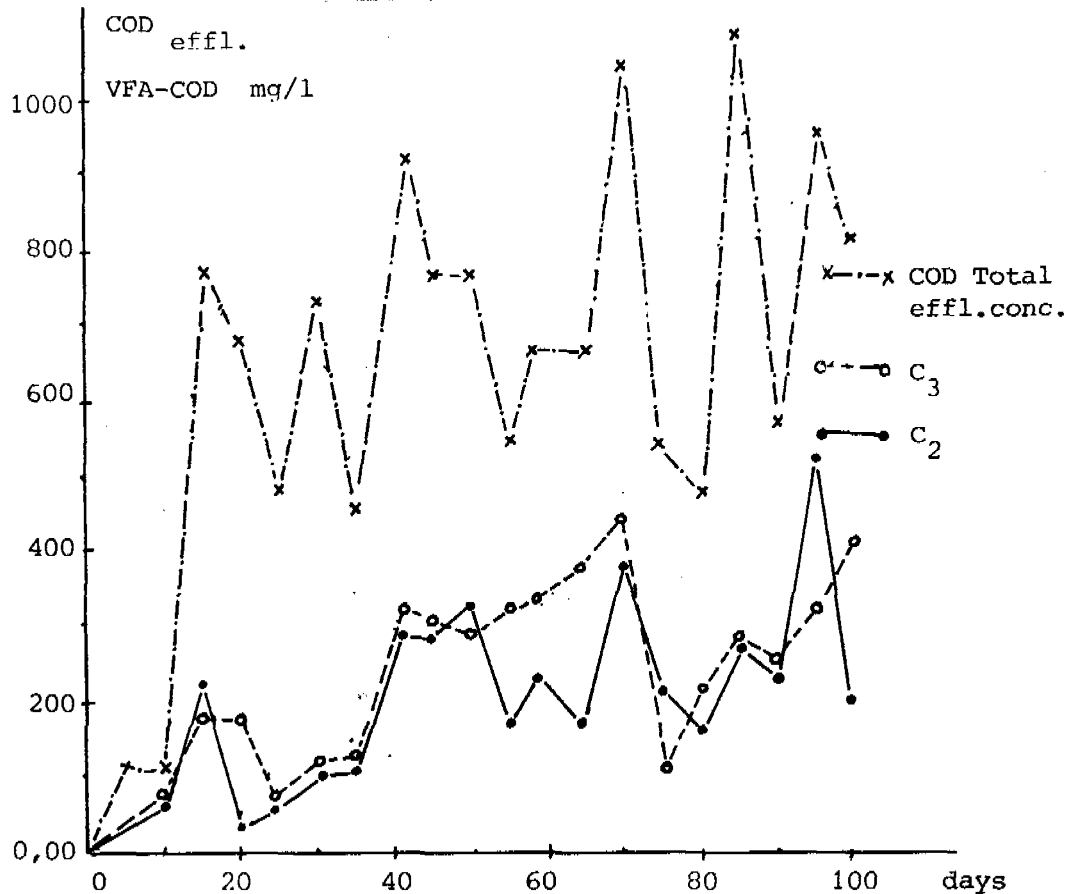


Fig.4.12 Total COD and VFA-COD conc. of the effluent Reactor B

Influence of the processtemperature during the start up of an UASB-reactor treating a medium strength synthetic wastewater.

Materials ands methods.

The experiments were done in UASB-reactors with a volume of 2.5 liter. The scheme of the equipment is given in Fig. A. Two reactors were applied during the experiements. One reactor was run at 30 °C, the second reactor was run at 38°C. The experiments were done in a temperature controlled room at 30°C. the reactor at 38° had a coat of a half flat tube, connected with a thermostate-bath ,kept at 38°C.

The seed sludge (digested sludge, spec. act. 0.12gCH_4-COD.$gVSS^{-1}.day^{-1}$) was supplied to the reactors in a concentration of 10 $gVSS.l^{-1}$. The compostion of the feed of both reactors is given in TABLE 1.

Table 4.6 Composition of the feed for the start up experiments

Component	Concentration ($g.l^{-1}$)
acetic acid	1.25
propionic acid	1.00
NH_4Cl	0.06
$(NH_4)_2SO_4$	0.015
KH_2PO_4	0.015
Yeastextract	0.100
traceelements according to Zehnder	1 ml/liter feed.

The starting up procedure is identical to the procedure which is described earlier with the succrose containing wastewater. To obtain an initial sludgeload of 1.5 g $COD.gVSS^{-1}.day^{-1}$, the initial flow to the reactors was 0.5 l/day. The feed was pumped into the reactors by a Gilson 6 way pump. The reactors were stirred every 30 minutes during 5 seconds (30 rpm) till the gasproduction exceeded 2.5 $liter.day^{-1}$. It is thought that the system was selfmixing from this point. The volumetric loadingrate (V_1) was increased with steps of 50-75 %, when the treatment efficiency exceeded 80-85 %. The treatment efficiency is calculated with the next equation:

$$n = \frac{(COD_{influent})-(COD_{effluent})}{(COD_{influent})} \times 100\%$$

Some parameters were measured daily:
- CH_4-production with a wet gasmeter
- pH of the effluent
- VFA concentration in the effluent by gaschromatography
- mediumflowrate by weighing

Regular determined parameters:
- $COD_{influent}$
- sludge examinations by microscope

sludge washout determined as : (COD-effluent)not centrifugated - COD-effluent centrifugated = washed out sludge (1.4gCOD= 1g VSS).

Results and discussion.

In Fig 1 and -2 the main results of the starting up experiment at 30°C are shown. The results of the reactor run at 38°C are shown in Fig. 3 and -4. Granules were detected after 40 days of operation in reactor R 1 (38°). In reactor R 1 (30°) some granules were seen after 80 days. When both reactors are compared it

appears that a much higher laodingrate and gasproductionrate can be reached at 38°, namely 2-3 times higher. Also the mean treatment efficiency is 5-10 % higer. In TABLE 2 the VSS-concentration in both reactors after ending the experiment are given, and also the mean diameter of the granules.

Tabl.4.5 VSS-concentration of the endsludge and mean diameter of the granules of reactor R1 and R2

	VSS-concentration ($g.l^{-1}$)	granule diameter (mm)
R 1	2.0	1.5
R2	5.8	2.5

The granules of both reactors consisted almost completely of a filamentous bacterium, resembling Methanothrix sp an acetoclastic methanogen. This bacterium has its temperature optimum at 38°C. The higheractivity of the sludge grown at 38° confirms earlier observationsthat the methanogens reactors are belonging to the species Methanothrix sp .

Conclusions

The start up of an UASB-reactor treating a medium strength synthetic wastewater is more favourable at 38° then starting up at 30^{0}. 2-3 times higher loadingrates could be reached at 38°.
Because the methanogens growing in the sludge in the reactor at 38° are kept at there optimal growth conditions, granulation is accelerated. In this way wash out of biomass is lower then when started up at 30°.

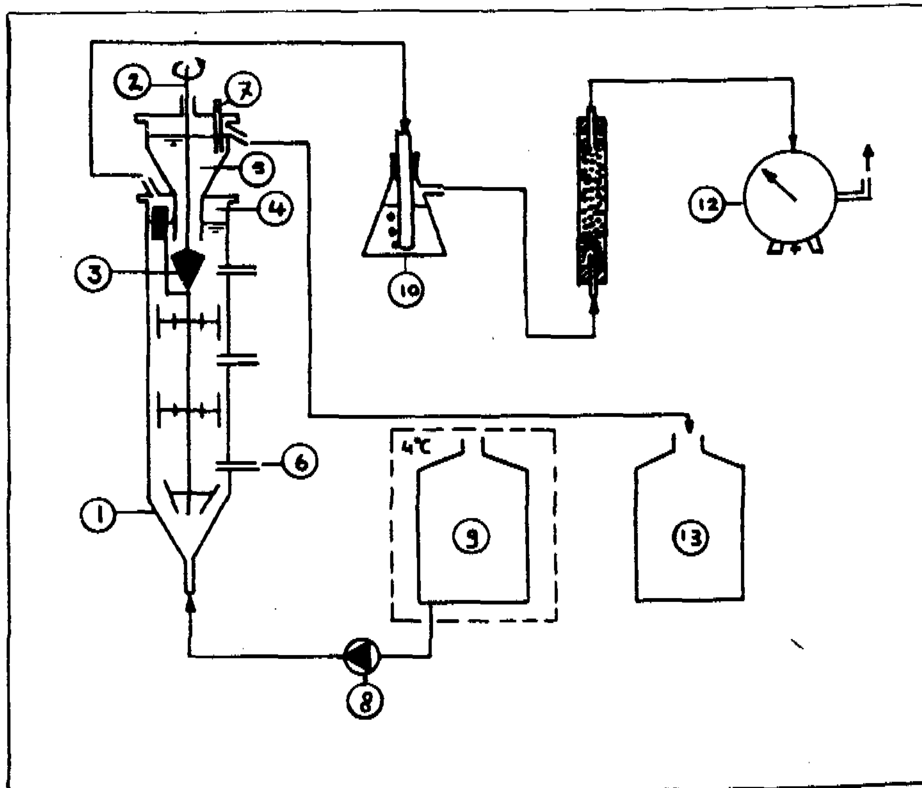


Fig.4.13. Equipment start up experiments at 30° and 38°C.

1. 2.5 l UASB-reactor
2. stirrer connected to a time puls clock
3. gas conductor
4. gas collector
5. settling compartment
6. sample ports
7. sample point effluent
8. Gilson feedpump
9. influentvessel
10. 5 N NaOH solution absorbing CO_2
11. Lime column ,, ,,
12 . wet gasmeter
13. effluentvessel

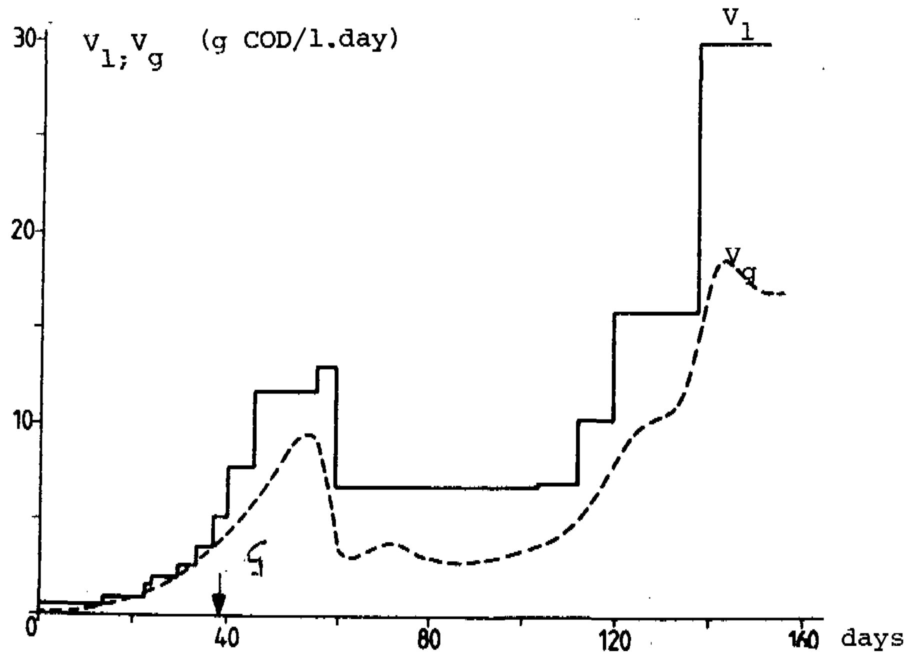


Fig.4.14. Coarse of V_l and V_g of the reactor run at 38 ^{0}C (R 2).

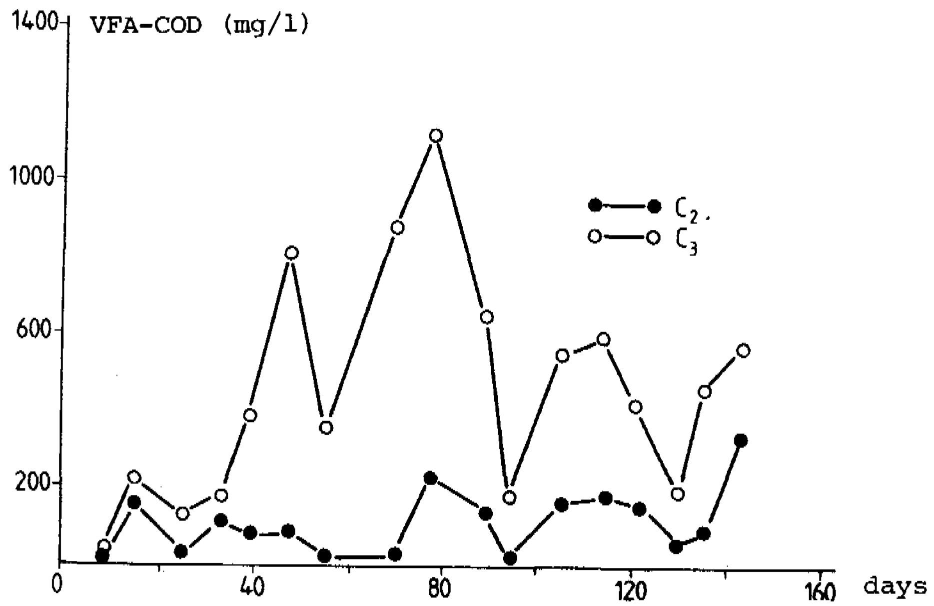


Fig.4.15. VFA-concentrations in the effluent of the reacor run at 38 ^{o}C (R 2).

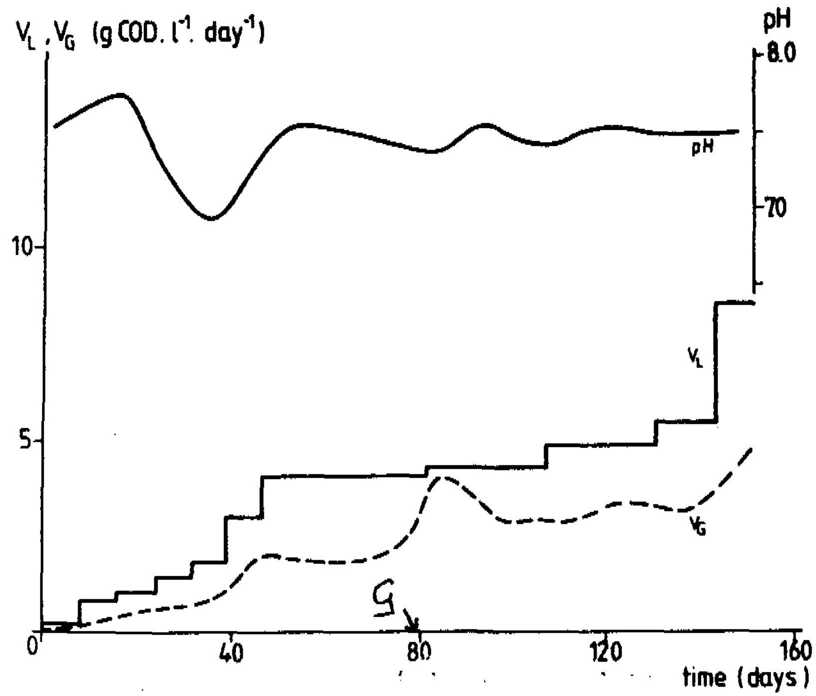


Fig.4.16. Coarse of the volumetric loadingrate (V_l) and the gasproductionrate (V_g) of the reactor run at 20°C (R 1).

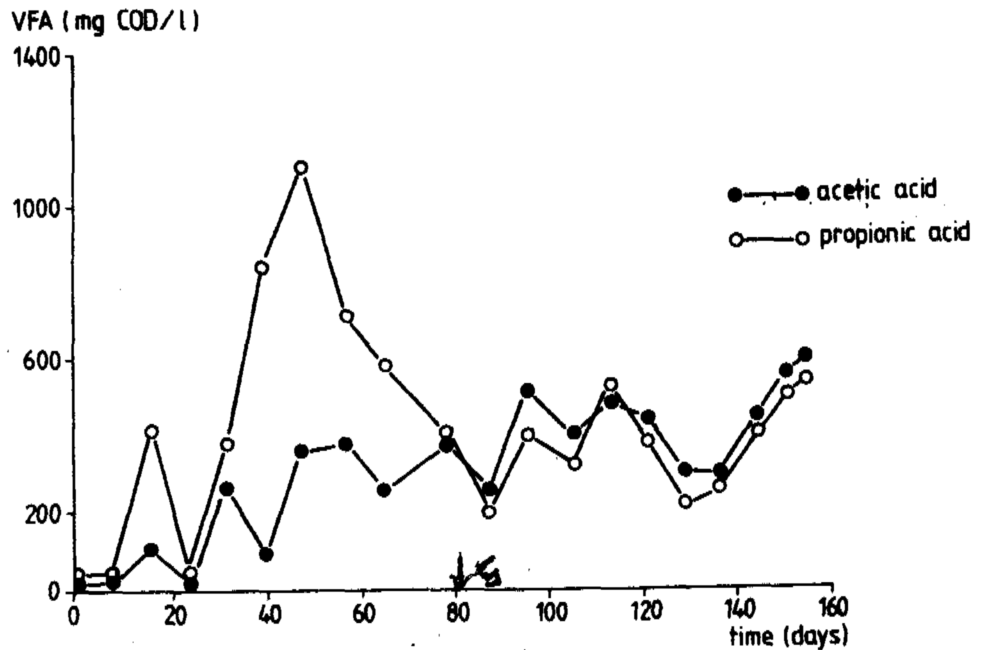


Fig.4.17. VFA-concentrations in the effluent of the reactor run at 30°C (R 1).

4.4. Instructions for experimental work on intermittent slow sand filtration

The purpose of the experimental work may be to find a suitable design for a filtration unit which is adapted to the locally available waste water. The most important variables in such research will be: available grades of gravel, sand or other possible filter media, the hydraulic and organic loading, influent pretreatment and filter maintenance. Also filter depth and hydraulic regime can be played with.

A typical filter installation is given in the figures and on the next page. A pebble-bed clarifier as described in paragraph 1.3.3. can be installed for prefiltration in order to remove some of the suspended particles (approx. 50%). Figure gives a schematic lay-out .

The filling of a column

The filling of the column is carried out by smoothly supplying the air-dry graded sand to the column. First some (5 - 10 cm) gravel has been put on the bottom as a support.
In order to obtain a good packing of the sand one has to knock on the wall during the filling or vibrate the column.
Then the column is filled with water by supplying it slowly from the bottom at a speed of 0.5 m/day.
In this way the air is expelled from the filter. After this the initial permeability of the column is determined. This permeability is the fluid velocity in the downwards direction.
Clean water is supplied at the top of the filter at such a velocity that the head stays constant. During the subsequent infiltration of waste water the permeability is measured regularly in order to follow the process of plugging.
The permeability is calculated as follows. At a constant head: the flow of the pump is measured. Assume a flow of 15.5 l/hr and a column diameter of 0.2 m , the velocity $v = Q/A = 0.0155 \times 24/ \frac{1}{4} \ (0.2)^2 = 11.8$ m/day.
As the permeability is a function of head the head at which the permeability was measured should always be mentioned.

In order to know whether the filter is well packed the divergence of the retention times is determined.For this purpose an inert salt(NaCl) is added to the filter feed (clean water) up till a known concentration. After some time this inert salt is left out again.The influent concentration is given in figure 4.13.

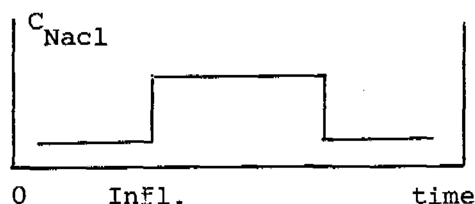


Fig.4.13. The influent concentration.

During the dosing of the inert salt the Cl^- -concentration is measured in the effluent.The form of the curve (concentration versus time) gives an indication of the quality of the packing.A sloping front indicates the presence of coarse canals:some influent passes sooner than the theoretical retention time.A long tail indicates disuniformity of the filter bed.Some parts of the solution remain in the filter for a longer time than the theoretical retention time.

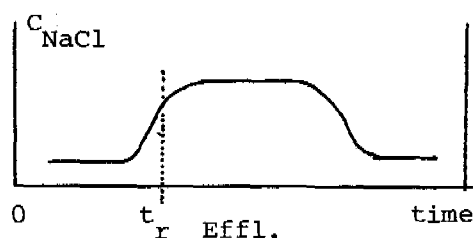


Fig.4.14.The effluent concentration.
t_r-retention time.

In order to check the improvement of the water quality by filtration various parameters in influent and effluent are compared.
The removal along with the height of the column can be monitored by analysis of samples tapped from different depths.
Analyses which are frequently carried out in filtration experiments are total suspended solids(TSS),turbidity,COD,BOD,NH_4^+ -N-concentration coliforms(Most Probable Number),Standard Plate Count and Dissolved Oxygen.
Temperature is also an important parameter .Higher temperatures usually lead to a sooner plugging of the filter.Prescriptions of the determainations can be found in chapter 3.

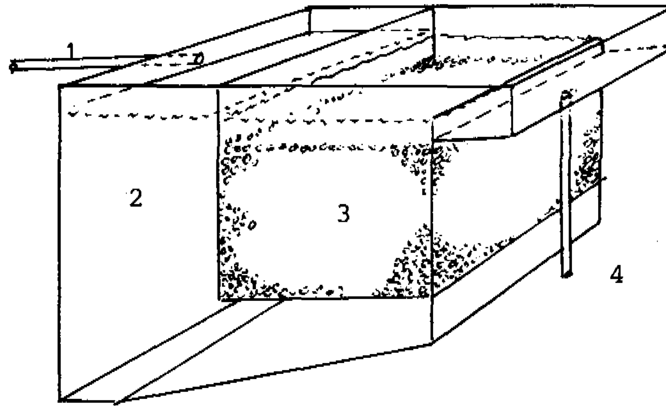


Fig. 4.15. The laboratory scale pebble bed clarifier;dimensions: bed depth:15 cm.Lenght: 35 cm.Width: 25 cm.Total volume:20.1 litre. 1.= influent pipe; 2= sedimentation compartment; 3= gravel bed; 4.= effluent pipe.

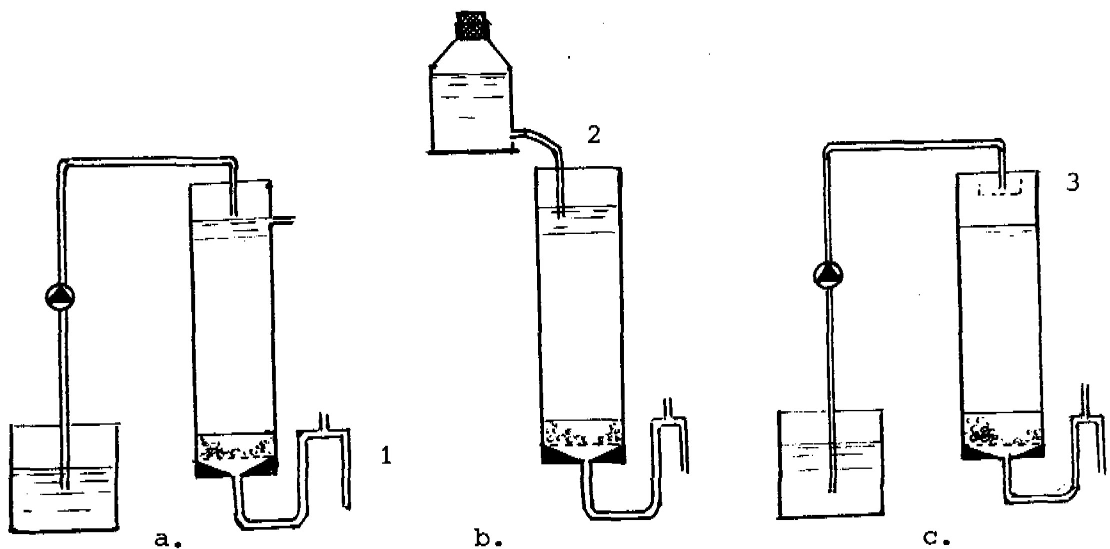


Fig.4.16.Laboratory scale filter units.

4.7.a.Installation for continuous filtration;

4,7.b.Installation for filtration with intermittent ponding;

4.7.c.Installation for intermittent sprinkling.

1.adjustable effluent tube

2.dosing siphon for constant head

3.sprinkling head

Flow regime
1.pumping period
2.drainage
3.dry period

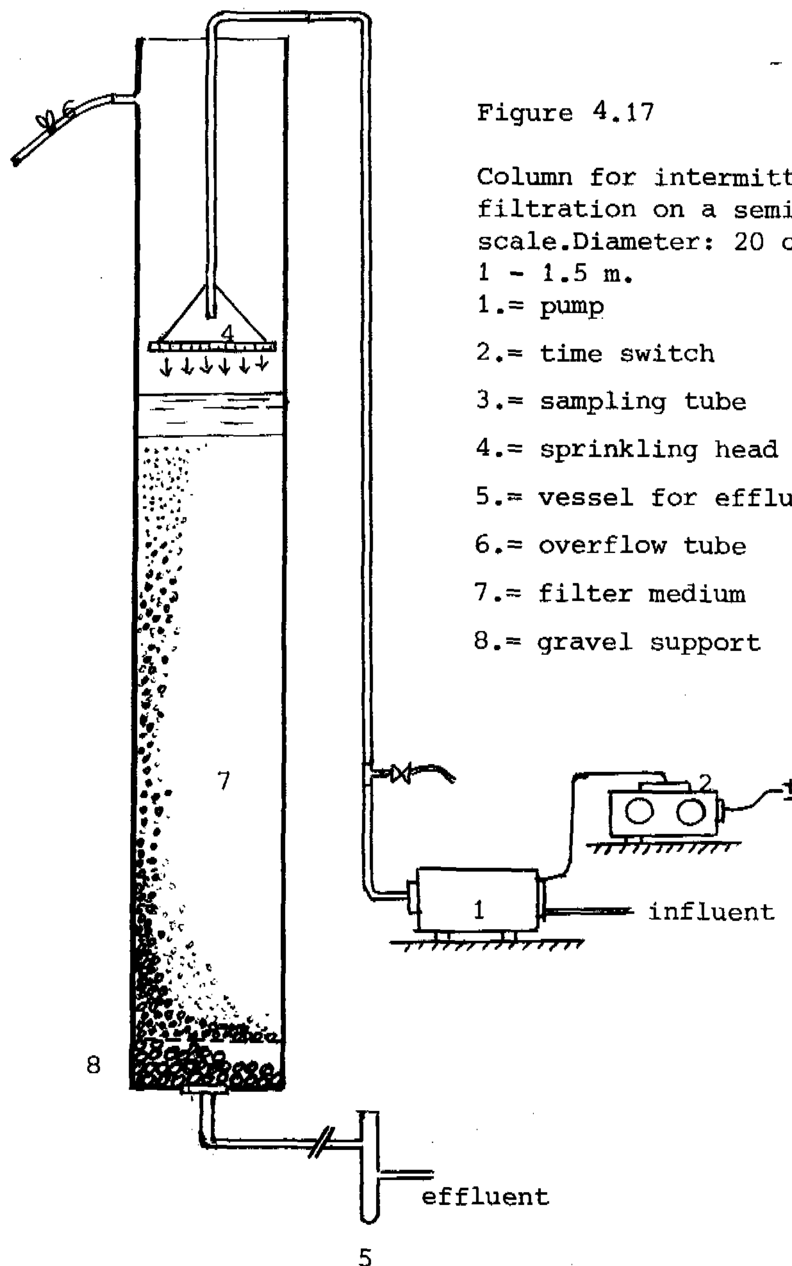


Figure 4.17

Column for intermittent sand filtration on a semi-technical scale.Diameter: 20 cm.Height of bed 1 - 1.5 m.

1.= pump

2.= time switch

3.= sampling tube

4.= sprinkling head

5.= vessel for effluent sampling

6.= overflow tube

7.= filter medium

8.= gravel support

Example of an experiment :the treatment of UASB-effluent with a slow sand filter.

This experiment was carried out in an installation *drawn in figure* 4.7.c.

The filter bed has an height of approx. 40 cm.It is filled with building sand.The grading of this sand was determined by sieve analysis. The following table shows the result of the analysis.

BUILDING SAND

Distribution of grain size

Fraction (mm)	Σ%
≦8,00	100
≦3,15	93
≦2,00	87,3
≦1,00	77,4
≦0,500	43,9
≦0,250	4,2
≦0,125	0,34
≦0,064	0,04
≦0,032	0,00

For this sand the effective grain diameter(D_{10}) is 0.31 mm.This means that 10% of the grains has a size smaller than 0.31 mm.This parameter can be derived from the above table.

The coefficient of uniformity $U = \frac{D_{60}}{D_{10}} = 2.0$

The bulk density (= weight of filter sand divided by the volume of the bed) is: 1.75 g/cm^3.The porosity is 33.8%.

The influent was anaerobically pretreated waste water from which a part of the suspended solids was removed by means of a pebble bed clarifier. The influent was pumped onto the filter during 2 - 3 hrs.The applied volume is approx. 1 m^3/m^2.day.

Because the permeability of the sand was bigger than the applied flow no ponding was obtained.

Table 4.4 shows the result.

The filtration of UASB-effluent through a sand filter (D_{10}= 0.31 mm).

TABLE 4.4. The filtration of UASB effluent trough a sand filter(D_{10}=0.31

Day nr.	Hydraul. load (m)	Pump. time (hr)	Infil. velocity (m/d)	INFLUENT COD (mg/l)	INFLUENT SS (mg/l)	INFLUENT O_2 (mg/l)	EFFLUENT COD (mg/l)	EFFLUENT SS (mg/l)	EFFLUENT O_2 (mg/l)	SS-inf. -effl. mg/cm^2
1	0,96	2	≈20	253	32		178	20		0
2	-									
3	1,00									1,2
4	0,63									2,8
5	0,70									3,8
6	0,86			298	44		214	25		4,9
7	0,86									6,5
8	0,88			226	42	1,2	154	25	5,8	8,2
9	0,84		12,1							9,6
10	0,75									12,8
11	0,75			364	88	1,6	221	30	1,9	15,5
12	0,75									19,9
13*	0,29			277	45	1,0	166	30	0,1	22,6
14*	0,69									23,0
15	0,55	3								24,2
16	-									
17	0,58									25,1
18*	0,71									26,1
19	0,58			213	37		97	18		27,3
20	0,57									28,4
21	0,57									29,5
22	0,13		12,7	256						30,6
										30,8

On day 13 Nitrogen and BOD determinations were carried out.

	Influent	Effluent
NH_4^+ -N (mg/l)	80	79
NO_2 -N (mg/l)	0	0.2
NO_3 -N (mg/l)	0	0.1
BOD_5^{20} (mg/l)	130	50

Comparison of the experiment with theory

In paragraph 1.3.3. equations were given to describe the plugging of filter with time as a function of the suspended solids loading(gr/m^2.day) (SSL).SSL can be calculated from the SS concentration :

$$SSL = \text{Daily hydraulic loading}(m^3/m^2.\text{day}) \;.\; SS(gr/m^3).$$

From the above table it can be calculated that the average hydraulic loading is approx. 0.75 m^3/m^2.day and the average SS-concentration is 48 gr/m^3. So SSL is 36 gr/m^2.day.

By interpolation from the curves represented by the following equations

$$\text{Days to plugging} = 2529(SSL)^{-1.733} \text{ and}$$

$$\text{Days to plugging} = 8859(SSL)^{-1.625}$$

for sand of a grain size of D_{10} = 0.17 and 0.40 mm respectively,it can be calculated the the expected filtration time to plugging is 26 days .

From the table is can be concluded that the expected and the found plugging time do agree rather well.After 22 days the column was not yet totally plugged.But the infiltration capacity had already dropped,which indicates the beginning of plugging.

5.FROM ENVIRONMENTAL PROBLEMS TO WATER POLLUTION RESEARCH

Technological research aims at changing a situation in society. Or more precisely:it provides us with techniques that help us to change a situation.If we see a water purification technologist (engineer) at work in the laboratory we would say at first:technology has to do with apparatuses:sedimentation tanks,filtration columns,microbiological reactors.But basically technology starts from a situation in society about which some,many or all people think there is a problem.
The scheme of figure 5.1. shows which different steps are taken to change such a problematic situation.
The frames indicate a certain activity and the arrows a logical relationship between two activities.

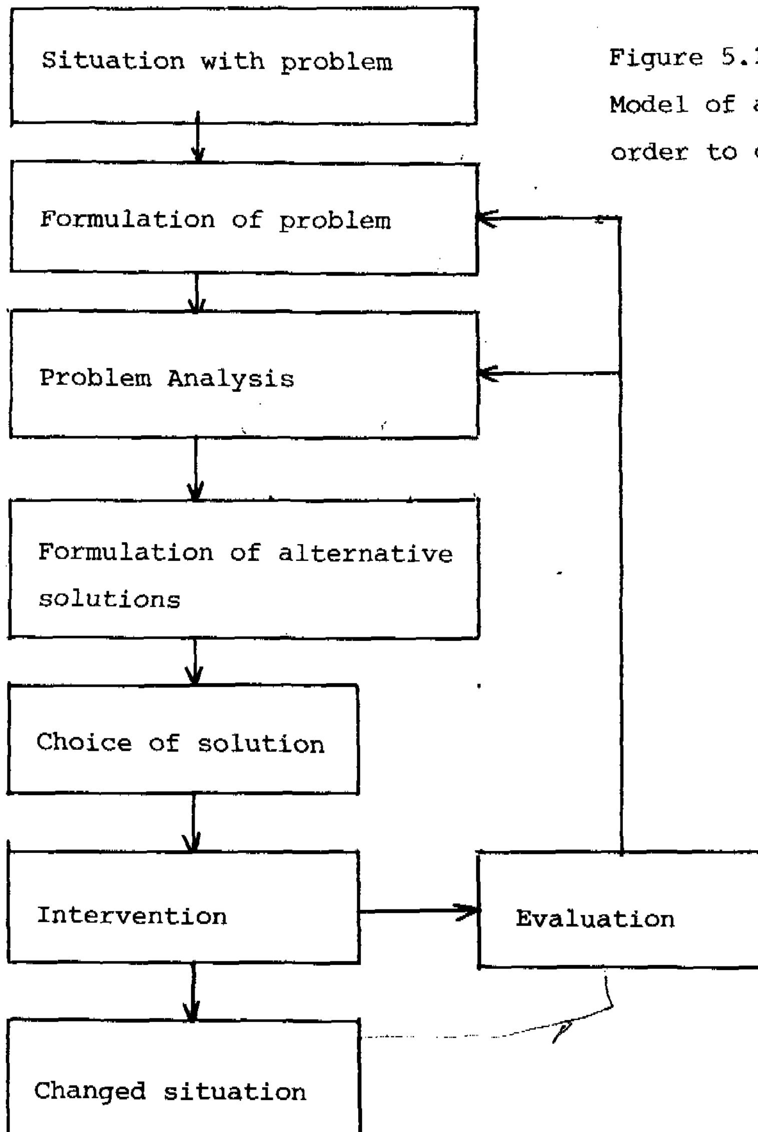


Figure 5.1.
Model of activities in
order to change a problematic situation

From this figure the role of science and technology on the one hand and the role of politics on the other can easily be derived.

One of the problems at which water technologists are working is the environmental problem of waste-water discharges into surface water.Whether a situation is called problematic and what people think the causes of the problem actually are is a matter of opinions,interests and values.

Let us take for instance the case of a region where the farmers see the yields of their crops ,and hence their incomes,dropping.

They also see that the water of the river from which they take their irrigation water has a bad quality:it smells,the water looks very turbid,fish are found dead.

The farmers come to think that the bad water is the cause of their decreasing harvests.They observe that nearby factories discharge a lot of very dirty water into the river and they go to the factories to complain about this.

The industrialists however deny the problem formulation by the farmers.They say:"your yields have dropped because there were insect pests.Insects ate your crops and then you applied pesticides and inadvertent use killed the fish in the river.And because your yields and incomes dropped the most skilled farmers sold their land and went away."Both parties have a different problem formulation.

Then the farmers go to the government.They ask:"we think that discharges of waste water are the cause of our problem.Do you agree? And if you agree,you have to make the factories do something about it, because we have this problem and moreover polluting the river is forbidden by our law".The government consults environmental scientists:"please, make us a problem analysis and formulate us (alternative)solutions". (See figure 5.1.).

As it is a situation with many aspects a team is formed,consisting of an environmentalist,a rural sociologist,an agronomist ,etc.

Their diagnosis could run as follows:

"The total of industrial effluents has a load of X kg/day organic substances, Y of ammonia.Z of heavy metals etc.

The effluents make the river water anaerobic.This kills the fish.

It is true that the farmers do not always handle their pesticides in the right way but this is not the cause of the fish mortality.

Anaerobic water results in anaerobic soils on which vegetables can not

grow.Although insects eat their part of the harvest and the skills of the farmers could be better,the bad water quality is a major hindrance to the improvement of the farmer's income".

In making the diagnosis (problem analysis) the scientists try to be as objective as they can.

As their terms of reference asked,the team also presents a set of alternative solutions.Their first solution is to change the production processes within the factories and provide them with treatment plants for their waste water.
The second one is a sewer to combine all waste water and pump it to the sea at some kilometres distance.
The third solution is to construct a treatment plant without change of the production processes.
The team also may have considered solutions like 'moving the farmers out of the region" or 'closing the factories' but these solutions they found politically unacceptable in the situation.

Which of the solutions will be chosen is not a scientific but a political question.Whether and how the question is solved depends on power relationships,the political system and governing social values.
It is possible that the report is laid aside and nothing is done to improve the water quality.Then,the farmers will be slowly pressed out of the area.
If something is done the environmental impact and the cost-benefit relationships will be important factors in the choice of alternatives.

Once the decision for one of the solutions has been taken the intervention takes place.In our example the intervention could be the construction of a water treatment plant.
The intervention basically leads to the desired change of the situation.
The problem of the farmers should be solved by now.
In reality along with the intervention a new problem could arise.
E.g. new factories are established in the area and the waste water treatment plant soon appears to be too small.
Such a thing becomes clear during an evaluation of the total series of

actions (after some time).From this evaluation everybody can learn.
The diagnosis can be carried out in a better way in the future and
it may be necessary to apply an additional intervention.

From figure 5.1.and the description above it can easily be seen that science and technology come in in the problem analysis and the proposal of alternative solutions.
Further,technology plays a role in the planning and design of the intervention.We can also distinguish two types of water purification research.
First,the research to analyse the causes of the bad water and soil quality.
Secondly,the research to develop effective waste water treatments methods.Research can be aimed at a particular situation:e.g. the water treatment at Tan Binh District.But also a more general approach is possible.E.g. the posttreatment of UASB-effluent not regarding a special geographical situation but aiming at the improvement of the method as such.This last type of research usually has a more fundamental character.

A model of action in research activities is given in figure 5.2.
A fundamental feature of natural sciences (which distinguishes them from a science like history) is that their theories and laws can not only explain phenomena,but they also can forecast.
E.g. "If I blow air into waste water under certain defined circumstances ,then the water will contain less organic substances after some days"
The scientific theories always have the character of :if .... then....
This forecasting is of course extremely important for engineers.
The aim of scientific research is to establish laws of this if...then...character.This is called the nomological character of natural science.
If one starts a research activity all information on the subject that is available from preliminary observation,from literature and other experts is collected.
Next the available data are ordered;already available observations are explained from the theoretical framework that already exists in literature.
Then, a hypothesis is formed.

The hypothesis is a forecast made from the available data. E.g.:"up to the grain size of 0.2 mm the removal of bacteria in slow sand filters is not dependent on grain size".
If the hypothesis is interesting enough for the advance of technology the next step (see figure 5.2.) is the testing of the forecasted facts by experiments.
From the experiments one may learn that the hypothesis was right or false.
If it is right,it is a confirmation of the more general theory from which the hypothesis was derived.
If there is enough confirmation for the general theory,the theory is considered as valid.
Before starting research to test a certain hypothesis it is useful to have the opinion of experts.They can be asked:
1.Is this a hypothesis which advances the field of science?
2.What do you think about the outcome?
A hypothesis is particularly interesting when experts disagree about the outcome of the planned experiment.

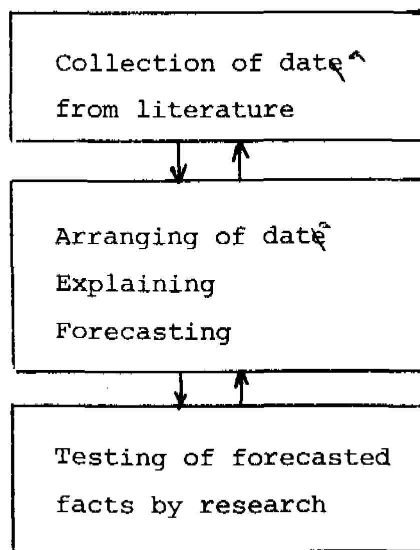


Figure 5.2.
Model of activities in experimental scientific research.

6. WRITING A SCIENTIFIC REPORT

As we explained in chapter 5 science can occupy itself with alot of questions and in a way that varies from one case to the other. Here we deal with the question:how to write a report about experimental work in the field of technology.
Anybody who has ever read a scientific article of this kind will have noticed that it is written according to a rather rigid structure.

The structure usually goes as follows:

.Title page with title,name of author(s),date,name of research institute, name of financing agency;
1.Introduction;
2.Aim(s) of the research;
3.Literature survey;
4.Theory;
5.Materials and methods;
6.Results of experiments;
7.Discussion;
8.Conclusions;
9 .Recommendations for further research;
10.Literature.
Index.
Because most articles and reports in experimental science are written in this way ,it makes it easy for the reader to find his or her way through it.
Now we will discuss shortly the contents of the chapters 1,2,3,4,5,6, 7,8 and 9.

Chapter 1.

Introduction.

This chapter should describe why the author found it useful to undertake the research.The chapter gives the social and scientific background of the subject:what problem in society the author wants to solve? And what is the scientific problem that makes the solution of that social problem difficult.For a deeper explanation see chapter 5 From a social to a scientific problem.

Chapter 2

Aim(s) of the research.

The aim of the research is to find an answer to a scientific question and in this way to advance technolgy in general or technology in a particular situtation:In Vietnam the aim of research is to apply existing knowledge about water purification to the special problems of (e.g.) Tan Binh District.From this broader aim the scientific problem of an efficient removal of algae from stabilisation pond effluent may be derived.

But although the remeval of algae by filtration is a technological subject with technological problems ,it is not yet a research question .

A research project should always start from an idea,an hypothesis about the possible outcome of the experiment.The experiment is set up to find an efficient removal of algae (for instance).The hypothesis could be that a gravel filter with a bed depth of 1 meter and a grain size of 4 to 10 mm can remove 90% of the algae from the effluent at an hydraulic load X.The aim of the research is to test this hypothesis. If the result of the experiment shows the the hypothesis is confirmed this is a strengthening of the exixting theory,The theory is useful as long as no experimental fact is found to falsify the theory.But as soon as only one fact contradicts the theory,the theory has to be revised.

Chapter 3

Literature survey.

All available relevant literature has to be used to find a theory about the subject of the research.

Mostly one starts with literature survey before any research is undertaken and from the survey the starting hypothesis (see above) is formulated.

But even if one knows that not all relevant literature has been studied it is useful to start experimental work.This brings with it that evident facts from literature will be found again.This may seem a waste of time and money.But it also has an important benefit.

Literature will be understood better after some experimental experience has been gathered.And it happens very often that one finds facts deviating from existing theories.

Even more important than literature survey is to discuss the research problem with other scientist involved in the field.

2 hours talk can be more useful than 2 months of literature survey.

The chapter on literature survey should describe the most important literature about the research question.

Chapter 4

Theory

From the broader literature survey in chapter 4. one specifiacally describes the mathematical theories about the research qustion. It can be sufficient to take 4 and 5 together with the emphasis on 5. This is particularly the case when already good literature surveys about the subject are available.

Chapter 5

Materials and methods.

This part describes as accurately as possible how the relevant analyses of water,sludge,soil etc. were carried out,what equipment and chemicals were used.This part is one of the most interesting to other researchers because the availability of a good method in literature saves a lot of research time.

Chapter 6

Results of experiments.

Here,the results of the different experiments are presented in figures and tables.Every figure and table should be provide with a short lucid text sothat even without reading the report it is clear what the figure/table is about.The results of every individual experiment should be preceded by a text abput the why and how of the experiment.It should also be clear why experiement nr 2 was following on exp. nr. 1.

Chapter 7

Discussion,

This part discusses the results and compares them with the existing theories. Differences with the hypotheses are well exponed and the researcher tries to explain the differences.If they cannot be explained from existing theories or from special circumstances during the experiment the researcer has to suggest a modification or extension of existing theory.Unexplicable facts should be exponed as well.It is no service to science to conceal well done experiments with difficult results.

In many cases Results and Discussion can be taken together into one chapter.After every experiment a discussion with theory is given.

(writing a report)

Chapter 8

Conclusions.

This chapter gives point by point the main conclusions from the research effort.First the conclusions about the hypotheses mentioned in chapter 2 may be mentioned.Then,one can make a list of all other important observations .

WAGENINGEN SEPTEMBER,1985

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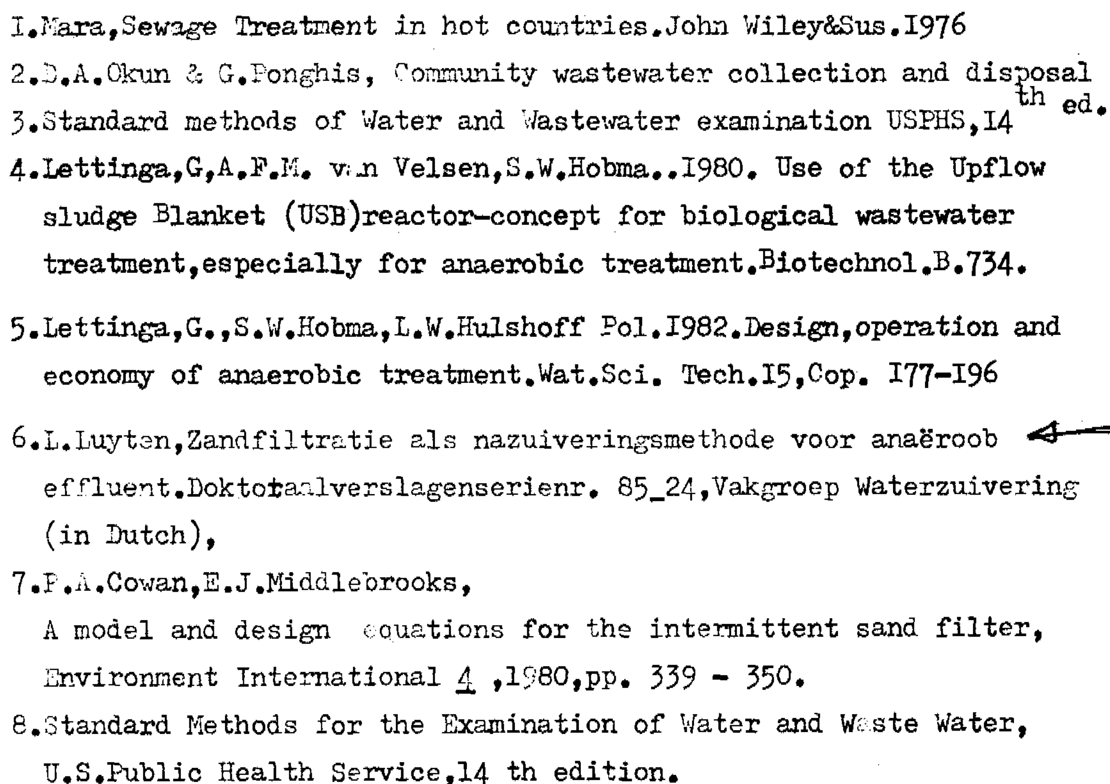

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