

Testing the Biodegradability of Organic Compounds

K. Wuhrmann and K. Mechsner

Enrichment of natural waters (fresh water and the sea) with synthetic chemicals or the products of their partial degradation constitutes a major human impact on aquatic ecosystems and, hence, represents a serious problem in water pollution control^{1) 2)}. Therefore, it is justified to request easy degradability of organic chemicals which may be expected to enter natural aquatic systems in the course of their use. Decomposition by the metabolic activity of aquatic organisms (mostly bacteria and fungi) is one of the mechanisms by which man-made organic compounds are eliminated from natural ecosystems.

Analytical and bioassay methods used for estimating such biological decomposition depend on the definition of biodegradability. From the ecological as well as from the water users' point of view it may be formulated as follows: "biodegradation is the elimination of a dissolved organic chemical from an aquatic ecosystem by the metabolic activity of the biocenosis actually present in this system. Final products of biodegradation are biomass and the oxides of the elements constituting the original compounds." For this definition to be ecologically useful, a supplementary statement on acceptable biodegradation rates is required. A reasonable rate parameter in complex systems is the half-life of the sum of concentrations of the original compound and its organic intermediates in the water phase. In natural waters a half-life of two days for organic compounds has been proposed on ecological grounds¹⁾. In artificial systems (waste treatment plants, bioassays) the half-life can be fixed according to specifications derived from local technical or experimental conditions respectively.

The above definition and rate requirement implies that biodegradable substances are utilized as substrates by a majority of ubiquitous microorganisms in soil or water, and that biodegradation starts without delay at nearly full rate (corresponding to the density of the bacterial biocenosis) when the compound is introduced into the ecosystem under discussion. Some initial

acceleration phase will, of course, always occur because of the species specificities in substrate utilization rates. Such rate differences automatically promote a dynamic adaptation of natural mixed populations to the new substrate (sociological adaptation)³⁾. Long lag periods in the attack of a compound by mixed biocenoses always indicate that an infrequent, specialized species is required for its decomposition.

conditions, inclusion of known compounds in any run and provisions for guaranteeing the statistical significance of the results are the normal additional requirements. Biototoxicity and degradability have to be neatly separated. This will perhaps require the investigation of extended dilution series. Aerobic as well as anaerobic degradation experiments are feasible. The OECD has in 1971 officially sanctioned such a simple aerobic test

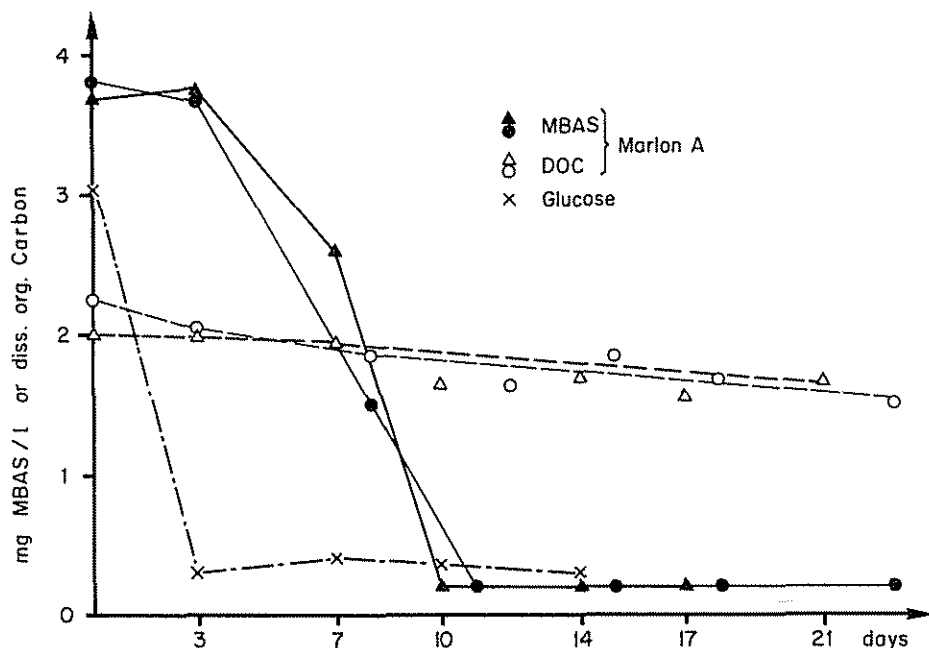


Fig. 1: Bioassay on the degradation of Marlon A. OECD «screening test». Experiments at two different times. For comparison: Degradation of glucose in parallel experiments. (DOC, mean values)
Determination of MBAS by the Chem. Dept. of EAWAG.

From the above considerations the principal requisites for an ecologically meaningful "biodegradability test" for pure compounds are easily deduced with respect to 1) the arrangement of the bioassay and 2) the analytical requirements for its examination.

1. Bioassay: on the grounds of the preceding discussion any test using a suspension of a mixed biocenosis of ubiquitous heterotrophic microphytes occurring in natural environments (e.g. top soil) is adequate. A conventional, buffered inorganic medium containing NH_4^+ as the N-source and the test substance as the sole source of carbon may be used. Standardization of

under the designation of "screening-test."⁴⁾

The bioassay based on a continuous fermentation system, as initially proposed by Husmann (adopted by OECD as "confirmatory test") is, in contrast to the above batch test, providing no kinetic information. Furthermore its result is mainly a function of the extent of sociological adaptation of the biocenosis in the system and depends, therefore, heavily on operation parameters such as sludge age. In view of the ecological information wanted, it is misleading, implies unnecessary methodical and analytical difficulties and is rather laborious.

2. *Analytical method*: curiously enough, "analytical" procedures in tests on the biodegradation of detergents have been restricted to reactions which are correlated with properties such as scum formation or surface activity. Examples are the methylene blue complex formation with anionic⁴⁾⁵⁾ or the Wickbold test⁶⁾ with nonionic compounds. The incompatibility of these "analytics" with the final aim of any biodegradation test is evident.

Unambiguous proof of biodegradability reduces the choice of analytical methodology to two concepts: 1) quantitative measurements of specific substances (the original compound and all its possible fermentation intermediates), or 2) quantitative observation of the elimination from the water phase of the original compound and possible intermediates by means of the overall parameter of dissolved organic carbon (DOC). The first concept generally will be infeasible with complex mixtures of compounds (e.g. commercial detergents). The drawback of the second is the loss of information necessarily involved when overall analytical parameters are used. In routine work, however, only the organic carbon method provides unambiguous, easily interpretable results⁷⁾. This is, of course, already known since long. Therefore, it is difficult to understand the recent officialization by OECD of the Wickbold test for determining the "biodegradability" of nonionic detergents. — It is unnecessary to indicate that bioassays examined by measuring the respiration of a mixed biocenosis

(BOD and other oxygen consumption tests) are also based on inadequate "analytics", since one mostly ignores the quantitative correlation of oxygen use and substrate consumption⁷⁾. Respiration measurements are hardly reproducible in routine work and would still not give the wanted information in a direct way.

Fig. 1 demonstrates with one example the contradiction between the decomposition and the seeming disappearance of an anionic detergent in the bioassay of the type of the "screening" test of OECD. The graph indicates clearly that the loss of complex formation with methylene blue (MBAS) is not at all related to elimination, since the dissolved organic C concentration decreases only very slightly within 21—23 days (compare the behaviour of glucose in the same test!). The compound in question is obviously far from being appreciably decomposed in the test period. It is disappointing that it happens to be the "soft standard" for "biodegradable" anionic surfactants!

Conclusion:

The choice of a *bioassay method* for investigating the concentration behaviour or the implications of an organic compound in a natural waterbody depends on the specific question to be answered. Concerning the problem of microbial breakdown, the most simple batch type tests are adequate. Bioassays based on continuous fermentation methods have to be rejected due to the uncontrollable bias intro-

duced by sociological adaptations of mixed biocenoses in such systems. The problem of *analytical methods* in routine tests on microbial breakdown is most trivial: evidently total dissolved organic carbon in the test medium is the only simple parameter available. Quantitative measurements of the original compound and possible metabolic products have, of course, to be used when the elucidation of the pathways and the kinetics of single steps in the decomposition reactions is required.

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Advanced Waste Water Treatment Studies at the EAWAG

M. Kavanaugh (Process Engineering Group)

Recent forecasts (1, 2) of future waste treatment loads (up to the year 2000) reaching surface waters in Switzerland have indicated that the present level of waste treatment (mechanical, biological and simultaneous chemical precipitation) will be insufficient for achieving desired specific water quality levels in receiving waters. Future water pollution control measures must include the requisite physical and chemical processes for additional removal of nutrients and refractory organic compounds.

Under Swiss conditions it is anticipated that three process alternatives will be considered for handling of municipal waste waters (wastes of both domestic and industrial origin):

- 1) Addition of physical-chemical unit processes to existing mechanical-biological treatment plants.
- 2) Chemical precipitation prior to biological treatment step with additional physical-chemical treatment steps as required.
- 3) Physical-chemical treatment alone.

PILOT PLANT FOR ADVANCED WASTE WATER TREATMENT EXPERIMENTAL LABORATORY TÜFFENWIES EAWAG/ETH

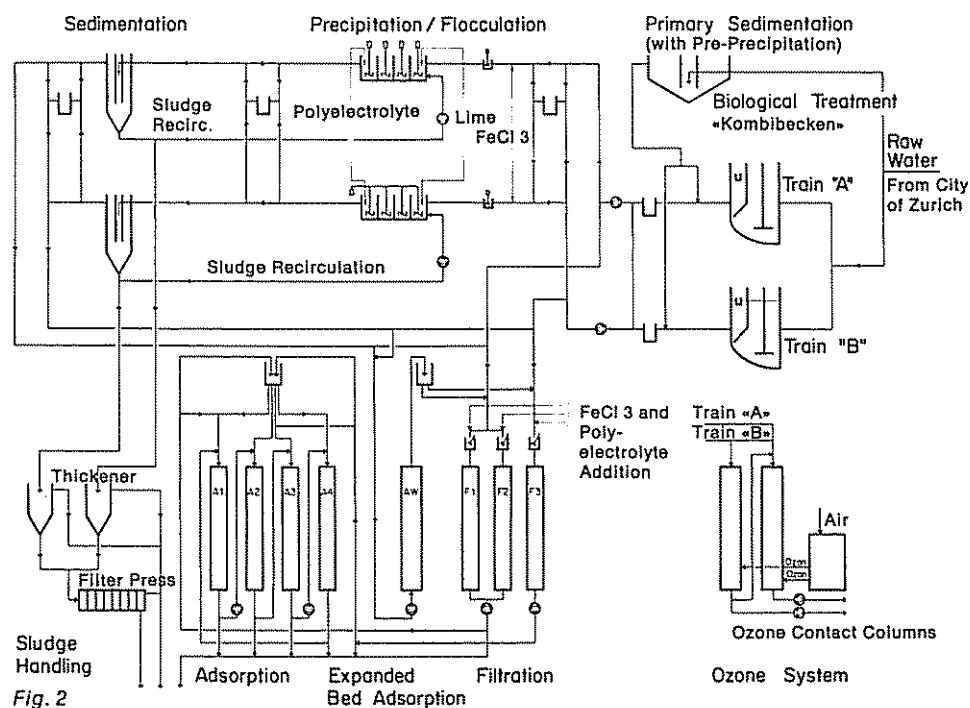


Fig. 2

2000, however, a significant rise in temperature during at least 90 days of the year must be reckoned with below Zurich's wastewater treatment plant.

2) Davos

The ski resort with its population of 10 000 has a maximum tourist capacity of 16 000 persons. During February

and March, when the peak skiing season brings the population to 26 000, the flow rate of the community's receiving body is also at its lowest with 1.3 m³/sec, thus resulting in a high pollutant concentration. This unfavorable situation will probably deteriorate even further by the year 2000.

Water quality tests focus on organic carbon (biogenic C, refractory C). At

present the average concentration for Feb/March is 9 mg/l; the admissible concentration is 4 mg/l. These values, however, reflect the present state: municipal wastewater is given no treatment. Advanced wastewater treatment (including primary and secondary treatment) on the other hand, would reduce the average concentration to 0.7 mg org C/l in the receiving body

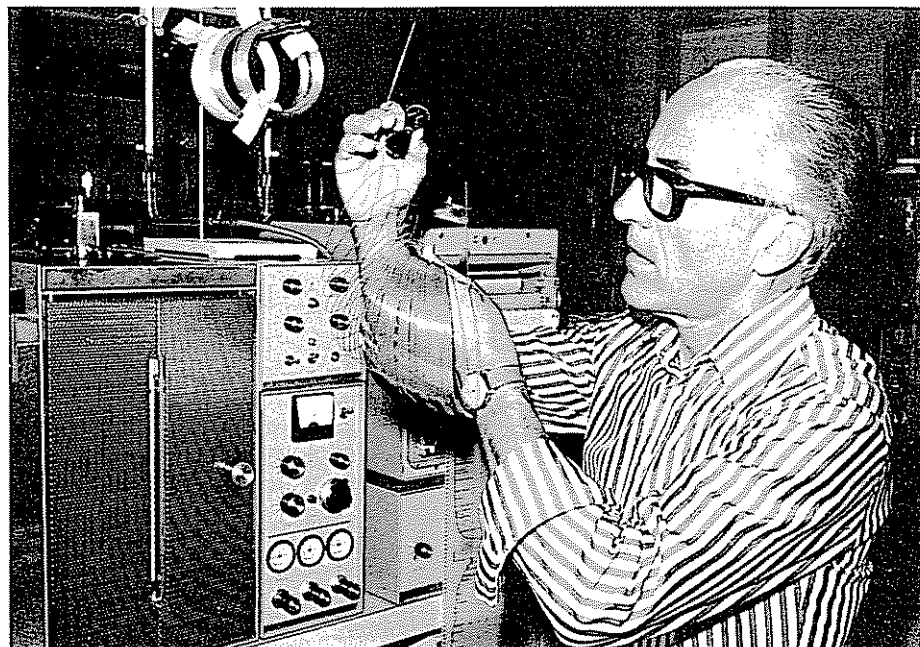
▶ Dr. Paul V. Roberts, the new head of EAWAG's Department of Engineering Science

Dr. Roberts was trained as an engineer in the United States; he received a Master of Science in sanitary engineering from Stanford University and a PhD in Chemical Engineering from Cornell. He has extensive practical experience especially in the field of unit operations for wastewater treatment and drinking water supply. During the past year Dr. Roberts has also been responsible for giving courses at the Swiss Federal Institute of Technology in Zürich.



▶ Professor Kurt Grob joins EAWAG and the Swiss Federal Institute of Technology.

Dr. Kurt Grob has been teaching at the University of Zürich and has established a laboratory for gas chromatography and mass spectrometry at our institute. He is a specialist on high resolution gas chromatography and has developed special glass capillary columns. Dr. Kurt Grob has worked out specific methods concentrating organic substances from potable water in unpolluted water; he has detected hundreds of substances in concentrations down to 10¹³ w/w and identified by GLC/MS. Last fall Dr. Grob has been honoured for his accomplishments with a honorary doctor's degree from the University of Berne. Dr. Grob gives courses on high resolution gas chromatography to the chemistry students at the ETH. His most recent publications are:



K. Grob: Organic Substances in Potable Water and in its Precursor Part I. Methods for their Determination by gas-liquid Chromatography *J. Chromatogr.* 84, 255—273 (1973).

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Authors: Dr. K. Wuhrmann is Professor of Applied Microbiology and Head of the Biology Section of EAWAG; Dr. Mechsner is a biologist in Dr. Wuhrmann's section. Dr. Kavanaugh is head of the process engineering group in the engineering department and coordinates the work at the advanced waste treatment pilot plant. Others participating in the work include M. Boller, J. Eugster, V. Krejci, P. Roberts, and T. Weber.

Editor: D. Stickelberger is the Deputy Head of EAWAG's International Reference Centre for Wastes Management (JRC/WHO).

Forecast for the Quality of Swiss Waters for the Year 2000

In 1973 55 per cent of the Swiss population was serviced by municipal wastewater treatment plants (primary settling and biological treatment); in a few years this figure will have been increased by a further 20%. Despite this effort, progressive deterioration of surface waters continues.

Water pollution control measures have until recently aimed at attaining a water quality similar to that in areas untouched by man; municipal wastewater treatment plants have been adequate in achieving this end. The discharge of industrial wastes has, however, brought a radical change. EAWAG has initiated a study on the effects of alternative water pollution control measures on future water quality.

The aim of the study was to improve water quality and also to determine the possible negative and positive impact of these measures on the economy, on urban planning and other fields of human activity. These findings are meant to provide decision-makers with a basis for the elaboration of a long-term water pollution control program. A preliminary*) study was carried out to ascertain the practicability of this very encompassing scheme.

The preliminary study includes three examples to demonstrate how forecasts can be made. The examples deal with:

- 1) a densely populated area with considerable industrial activity likely to increase even further (Glattal)
- 2) skiing resort without industry (Davos)
- 3) mountain lake in a conservation area with no residential population in the drainage area (Tomasee)

The following is a summary of the results:

1) Glattal

The 36 km long Glatt Valley, through which a river of the same name flows, cuts through the north eastern part of the agglomeration of Zurich. The catchment area covers 240 km². The Glatt River is an outlet of the Greifensee. Although the Greifensee, which has 5 affluents, a surface area of 8.5 km², a catchment area of 160 km² and a mean depth of 19 m, belongs to the Glatt Valley, it was dealt with separately in the study.

The *Greifensee* is highly eutrophic. While eutrophication sets in at 2.5 tons P/year, the phosphorus load for 1970

Water Pollution Control Alternatives Fig. 5 to 8 (k = tolerance threshold)

0: No wastewater treatment plant. No other measures (Davos).

1A, 1B, 2A, 2B combinations of the following:

A Per capita P mass emission rate 4.2 kg/yr (1970: 1.7 kg/yr).

Total industrial load coefficients rise in relation to the productivity increase. Remaining total load coefficients (TLC) as in 1970.

B Per capita P mass emission rate 0.6 kg/yr.

Industrial TLC 10% of A. Remaining TLC as in 1970.

1 Primary and secondary treatment for 100% of the wastewater and surface run-off from residential areas. In lake drainage areas additional precipitation.

2 Primary and secondary treatment, precipitation and adsorption for 100% of wastewater and surface run-off from residential areas.

3 Transfer of wastewater via ring trunk sewers (Greifensee).

Fig. 5: Greifensee: Development of the P load.

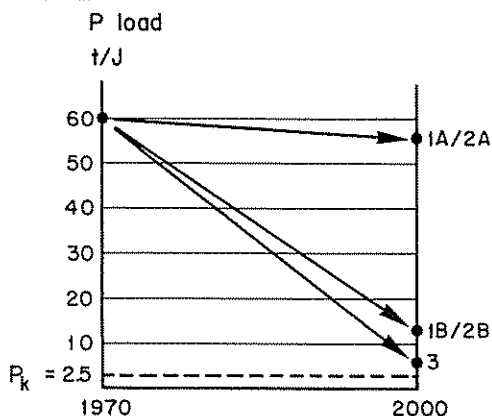
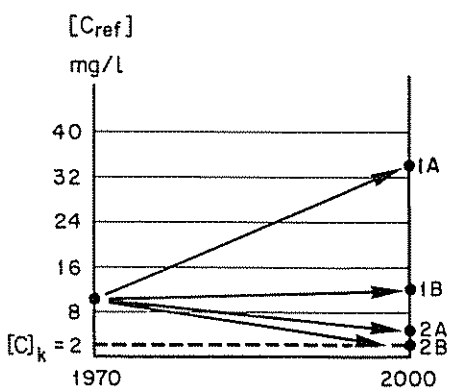


Fig. 7: Glatt: Development of the refractory carbon concentration.



was found to be 60 tons. 55 per cent of this load stems from detergents and human excrements, 30% from light industry. The remaining amount comes from various sources, mainly agriculture.

1) Assuming that the detergents are void of phosphorus, and domestic wastewater and run-off are given primary, secondary and tertiary treatment, there would still be a P load/year of 13 tons in the year 2000, i. e. five times greater than the admissible tolerance level.

2) Assuming that all wastewater were kept from the lake's drainage basin by means of a ring trunk sewer, the phosphorus load of 4 to 6 tons/year reckoned for the year 2000 from sources such as agriculture would clearly exceed the tolerance threshold (fig. 5).

Another critical source of pollution is refractory organic carbon from industry and street run-off. By the year 2000

Fig. 6: Greifensee: Development of the refractory carbon concentration.

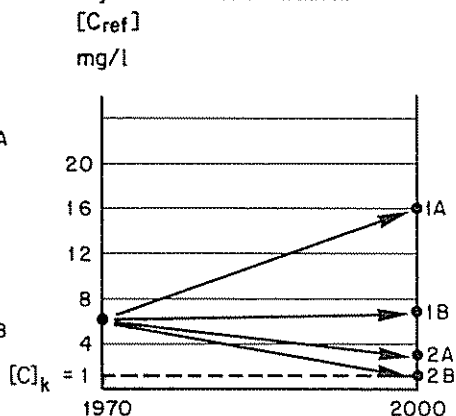
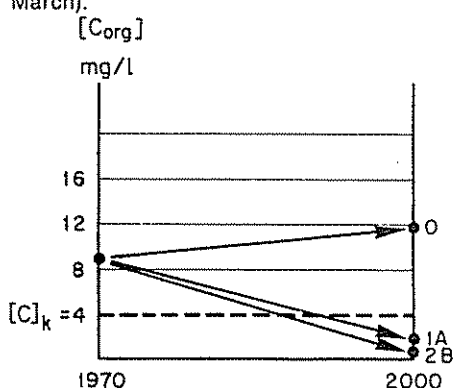


Fig. 8: Natural water bodies: Development of the organic carbon concentration (Feb./ March).



the most advanced pollution control measures including in-plant treatment, mechanical and biological treatment, precipitation and adsorption for all types of wastewater will be necessary to stay below the critical concentration level of 1 mg C_{ref}/l in lakes (fig. 6).

In the *Glatt River* the average tolerance threshold of 2 mg C_{ref}/l is exceeded by 500 per cent (fig. 7). Measures similar to those for the Greifensee will be necessary to stay just below the tolerance level by the year 2000.

Thanks to strong aeration from the atmosphere the Glatt is expected to remain well aerated even under unfavorable conditions.

Heavy metals can be kept below critical concentrations with advanced treatment; with only mechanical and biological treatment, short-term peaks must be expected.

Today's thermal pollution of the Glatt does not exceed 3°C. By the year

*) Original title: «Gewässerschutz 2000 — Prognose des Zustandes der Gewässer in der Schweiz bei alternativen Gewässerschutzmassnahmen». Documentary report Sept. 1973. The preliminary study was carried out by EAWAG and the planning office Basler & Hofmann, Zürich.

Pilot plant scale investigations of these three process alternatives are presently underway at our Tüffenwies experimental station. The overall objectives of these studies are:

- i) to demonstrate the technical and economic feasibility of various combinations of physical and chemical unit processes for advanced waste water treatment, and
- ii) to determine design criteria and the range of optimum operating conditions for the individual processes.

The pilot plant, a flow sheet of which is shown in figure 2, consists of two parallel treatment trains with a maximum hydraulic capacity of 0,4 l/s in each train. The facilities include precipitation and flocculation reaction tanks, sedimentation in a sludge blanket clarifier, downflow filtration columns, fixed and expanded bed adsorptions columns, ozonator and ozone contact columns, and thickeners plus a filter press for solids handling.

The first experimental series were directed at a thorough investigation of phosphorus removal by post precipitation following biological treatment in a "Kombibecken" (activated sludge aeration and sedimentation combined in one tank) with the major objective of determining the optimum chemical and physical conditions for precipitation, flocculation, and sedimentation. The parameters investigated initially included type of initial mixing device prior to the flocculation basin (static mixer, continuous stirred tank reactor, and no mixer), pH (7 and 8,8), iron to total phosphorus mole ration ($Fe/P = 1, 2, 4$). In the second sequence of experiments, the surface loading rate in the vertical sedimentation tank (approximately 1.5, 2.5, 3.5 and 4.5 m/h), and mixing conditions in the flocculation basin (residence times of 3, 5, 8 and 15 minutes, one and four cells in series, and energy input, measured by mean velocity gradient, of approximately 15, 60, and 120 (seconds⁻¹) were studied.

A factorial design permitted an analysis of variance of the effects of the above parameters on removal of dissolved and total phosphorus, suspended solids, dissolved and total organic carbon (DOC and TOC) and COD.

Results of the first test sequence indicated that the type of initial mixing device had no statistically significant effect on the removal of total phosphorus or organic carbon. Within a 95% confidence limit, phosphorus precipitation at a pH of 8.8 demonstrated superior removals of total phosphorus and total organic carbon, apparently due to superior performance of the sedimentation basin at the higher pH values. The effect of pH on the removal of dissolved phosphorus was statistically insignificant. At either pH level, and $Fe/P > 1$, removals of dissolved (DOC) and total (TOC) organic

carbon were, respectively, 5 to 30% and 20 to 60%, which corresponds to the additional organic carbon removals that can be obtained in post precipitation phosphorus removal.

In the second test sequence, in which chemical conditions were held constant ($pH=8.8$, $Fe/P=2$), it was clearly shown that the solids/liquid separation step, in this case, a sludge blanket clarifier, controlled the efficiency of the process for removal of total phosphorus and total organic carbon. A flocculation residence time of about four minutes was sufficient to produce easily visible flocs, ranging in size from 50 to 400 μ . Although floc break-up was observed at the highest mean velocity gradient ($G=120 \text{ sec}^{-1}$), this had no statistically significant effect on removal because of the efficiency of the sludge blanket clarifier. When the surface loading rate (upflow velocity) in the clarifier was less than 3 m/h (1730 gpd/ft²), total phosphorus

removal was greater than 80%, regardless of the mixing conditions, with a suspended solids concentration in the effluent of less than 15 mg/l. The solids concentrations in the sludge blanket was greater than 600 mg/l at this surface loading rate. TOC removals ranged from 20 to 50%, again irrespective of mixing conditions in the flocculation basin.

Studies on granular media filtration, both as a suspended solids polishing step, and as a substitute process for postprecipitation, replacing the precipitation / flocculation / sedimentation process sequence, are currently in progress. Future unit process investigations will include optimization of organic carbon removal by activated carbon, chemical oxidation, and disinfection with ozone, precipitation and solids handling by thickening and filtration. The final stage of the program will involve comparisons of the various process combinations described.

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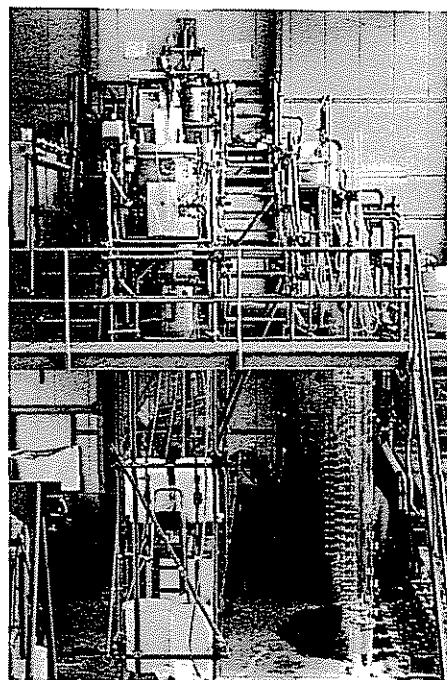


Fig. 3: Overview of Pilot Plant for Advanced Wastewater Treatment. Filter and Carbon Columns on right side, Flocculation and Sedimentation Units in center. Biologically treated wastewater from Zürich is treated by physical-chemical methods for removal of phosphorus, suspended solids and refractory organic compounds.

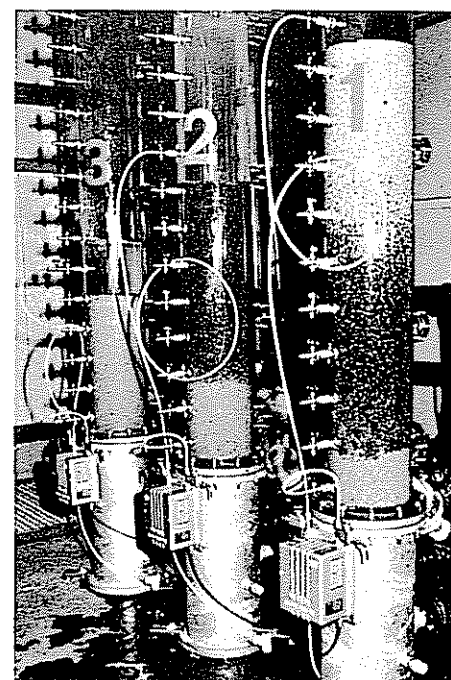


Fig. 4: Multi-media Filter Columns. A comparison of several multi-media filters will permit optimum design of this essential unit operation for suspended solids removal in advanced wastewater treatment.

during February and March (fig. 8). Even if only mechanical and biological treatment is provided for a maximum population of 35 000, the organic carbon concentration could be reduced to 1.6 mg/l during Feb/March. Yet, excess concentrations cannot be prevented during short-term drops in the stream flow and the frequent wastewater peaks.

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3) Tomasee

The Tomasee which lies 2445 m above sea level, has a catchment area of 2.0 km² and a surface area of 2.5 ha; it is 10.2 m at its deepest point. The only possible source of pollutants besides erosion and the atmosphere is cattle, wildlife, tourists and fish. The lake's good water quality is borne out

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by an oxygen content of 9.5 mg/l and a DOC of 0.4 to 0.6 mg/l.

If climate, water economy and pollutant load from the atmosphere remain the same, the water quality in the year 2000 will be as good as today's.

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