

RESEARCH PROJECT COMPLETION REPORT
OWRR PROJECT NO. A-041-OKLA.

EVALUATION OF WATER QUALITY BY MEANS OF
ENZYMES AND OF 'THRUFLO' ANALYZERS

Submitted to

The Oklahoma Water Resources Research Institute
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GRANT A-041-OKLA: EVALUATION OF WATER QUALITY BY MEANS OF ENZYMES AND OF
"THRUFLOW" ANALYZERS

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SUMMARY

- 1) The possibility of using enzymes in the determination of water quality has been evaluated, with respect to three specific problems: (a) pesticide residues; (b) organic impurities; and (c) oxygen.
- 2) Methods for the determination of pesticides with acetylcholinesterase have been surveyed and evaluated. Although many procedures have been described that are based on this principle, there appears to be scope for further improvement. However, other applications of enzymes appear to be more promising.
- 3) Specific organic substances, such as glucose, can be easily determined by enzymatic methods. However, because of their specificity enzymes are not well suited to replace tests like COD and BOD, which determine diverse oxidizable materials.
- 4) Improved procedures have been developed for the COD determination. They include: modification of the procedure to analyze small samples in ordinary test tubes with no condenser; construction of a special heating block to accommodate several samples; and determination of the dichromate consumed by spectrophotometry.
- 5) Several oxidase enzymes exist, that could be adapted to the determination of oxygen in water. However, they do not offer a great advantage over the conventional Winkler test.
- 6) A system has been developed for information storage and retrieval, that is quite simple yet efficient and versatile. It can readily be applied to the burgeoning literature concerning water resources. The information is stored in typewritten or other convenient form. IBM cards are used to produce an author and a classified subject index. The classification scheme is hierarchic and designed to be expanded and/or modified as the collection grows.

REPORT

Enzyme-Catalysed Reactions for the Evaluation of Water Quality.— The chief consideration which commends the possible use of enzyme-catalysed reactions in the evaluation of water purity is that catalytic methods of analysis are in general much more sensitive than conventional, equilibrium methods (1).

Since the enzyme catalyzes a reaction which is, in any case, spontaneous,

the desired signal is provided by the free energy of the latter, while the enzyme serves as a "valve". Thus the signal is amplified.

A similar situation exists in bioassays. Here the living organism acts as the amplifier -- it can take a weak signal, such as a low concentration of some toxic agent, and convert it into an easily observed biological effect. The energy for amplification is in this case provided by metabolism, which in the absence of toxic agent keeps the organism functioning "normally".

These considerations point up the existence of a close relationship between enzyme-catalysed analyses and bioassays, that might lend itself to exploitation.

Another general characteristic of enzymic catalysis is specificity. This is advantageous if one desires to determine a particular substance, e.g. glucose, in a multi-component mixture of uncertain composition.

Specificity is however a disadvantage if one wishes to determine a group of substances, as is done by the COD and BOD tests. Since these tests have serious shortcomings, it has been a part of our original plans to search for enzyme-catalyzed reactions that might be used in their stead. However, the search for a suitable enzyme was not successful. It was therefore decided to develop the program in other directions, as described below.

Improvement of the COD Test.-- The standard procedure (2) is relatively time-consuming and cumbersome. The following modifications were introduced and tested.

Two milliliter samples are used, with 5 ml of dichromate-sulfuric acid reagent. The reaction is conducted in ordinary 16 x 200 mm test tubes. They are placed in a specially constructed heating block, that is maintained at 150 ± 1.5 °C. The test tubes are loosely stoppered by a glass marble placed over the mouth

and no condenser is necessary.

After reaction, the mixture is cooled and diluted volumetrically with water. The absorbance of the solution is measured at 350 nm with a spectrophotometer (Coleman Junior II or equivalent) and compared with that of a control.

The dichromate reagent is made up volumetrically from reagent-grade sodium dichromate, and it can serve as primary standard. If dilution of the control gives results in accordance with Beer's Law no additional calibration is necessary. This was the case for the instrument used in our experiments; if necessary, a correction can easily be made. Optimum precision is obtained if the absorbance is between 0.8 and 0.2.

Samples with a higher COD may be diluted prior to testing. Low COD samples may be measured with no loss in accuracy by using a lower initial dichromate concentration and then reducing the final dilution.

With a glucose test sample, this procedure gave the same results as the standard method, with a standard deviation of 0.7%.

Non-Oxidizable Impurities.-- Many organic substances do not react with dichromate in the COD test, or consume less than the equivalent amount in the 2-hour reaction period conventionally used. Examples are benzene and saturated hydrocarbons.

Many experiments were done to determine whether changing the reaction conditions and/or adding diverse compounds in small quantities would promote the reaction. With hydrocarbons, none of the tests were successful.

With glucose and many other substances, the 2-hour reaction period is longer than necessary. In the procedure described above, in which reaction temperature and other factors can be closely controlled, a 30-minute heating period is sufficient.

Determination of Pesticides with Acetylcholinesterase (AChE).--- Many commonly used pesticides inhibit the activity of the enzyme AChE, and many have utilized this fact as a means of determining pesticides. In 1970, a procedure for the determination of pesticides with AChE was included in the "Official Methods of Analysis" (OMA) (3), and this is an important advance, because OMA procedures are subjected to critical appraisal and cooperative testing. However, the OMA method falls very short of being the optimum application of the principle.

In the OMA method, the pesticide-containing sample is mixed with an AChE preparation and the mixture is allowed to react for a fixed length of time with acetylcholine. The remaining acetylcholine is then determined by first converting it into hydroxamic acid and then reacting the latter with iron(III) ion, which forms a colored complex; finally, the color intensity is measured with a colorimeter.

This method of determining AChE activity is unquestionably more complicated and laborious than others which have been described in the literature. One difficulty is that the pertinent literature is quite extensive and scattered -- a recent review of methods for determining AChE activity lists more than 300 papers (4). It seems apparent that the OMA method was developed without making a critical comparison of the alternatives, and that if such a comparison were made likely some other procedure would be found preferable.

Another difficulty has to do with the AChE reagent. The OMA method employs enzyme isolated from bovine erythrocytes. Since the isolation is not easy, the quality of the product is open to question. But no specifications of the latter have been provided.

Part of the work done in the project was to test the OMA method. As the difficulties mentioned above became evident, it was concluded that the method was not a good one, and that alternatives should be sought.

We tested experimentally the possibility of measuring the rate of acetylcholine hydrolysis with a pH meter. However, the apparatus needed, and the procedure employed, were judged to be too cumbersome for practical use.

We then tested another modification. The acetylcholine was mixed with an indicator, bromthymol blue, and then with AChE or with AChE plus inhibitor. When sufficient acetic acid had been formed by hydrolysis of the acetylcholine, the indicator would undergo a certain color change, and the time needed to develop the change was measured. The length of time would of course increase with the amount of inhibitor present.

The measurement could be made with very simple apparatus, hence the method might be suitable for field determinations.

However, the initial pH of the samples being tested had a critical effect on the results. In practice, it would prove very difficult to control this variable.

All in all, the experimental tests were not as favorable as had been anticipated, and the investigation was not pursued further.

The results of the extensive literature search made in connection with this part of the project will be published as part of the M.S. Thesis of Mr. M. L. Alden, which is now being prepared.

Determination of Dissolved Oxygen in Water.-- Dissolved oxygen plays a vital role in the biology and ecology of aquatic systems, and therefore its determination is of basic importance. Although methods for this determination are legion, they leave something to be desired with respect to convenience and specificity.

Some experiments were done to modify the standard Winkler method (5) so it would be more rapid and convenient. The concentrations of the reagents were adjusted, and their volumes reduced, so the test could be done in a 25-ml syr-

inge, into which the sample to be tested could be drawn directly. The preliminary results obtained were promising, however adequate testing could not be completed before the end of the project.

We also considered the possibility of developing a test for oxygen based on its reaction with a suitable substrate, catalyzed by an oxidase enzyme. Several such enzymes are known, and it may be anticipated that any one of these reactions would be less susceptible to interferences than conventional chemical methods.

A promising possibility is glucose oxidase. At present, this enzyme is widely used in the determination of glucose in blood. With excess glucose, the reaction could be used to determine oxygen. Unfortunately, it was not possible to test this idea before the end of the project.

Efficient, Versatile System for Information Storage and Retrieval.-- The scientific literature has grown to immense size and is still increasing at a very rapid rate. The difficulty of retrieving information from the literature has been increasing apace. As a result, investigators must devote an increasing proportion of their time to that activity, even so they are likely to miss significant developments. Finally, this causes wasteful duplication of effort and diminished the effectiveness of ongoing research.

The field of water resources is, in this respect, worse than many others, because research activity has been greatly increased in the recent past, with a concomitant increase in the amount of primary data. At the same time, the field is not well served by a body of secondary literature -- abstracts, reviews, treatises, and tabulations of data. Some interval must necessarily elapse between the time when new research results are first reported and when they are effectively incorporated into the logical framework of the subject. It is fair to say that, in the recent past, a glut has developed; to relieve it, it is desirable to

develop novel and effective methods for information storage and retrieval.

A simple, versatile, yet efficient method has been developed, which may be used by an individual researcher to classify and index a reference collection. The principal features of it are summarized below.

As the collection grows, a classification is developed for it, which is hierarchic and decimal, i.e. at each level, there can be up to nine classes, each of which can then be subdivided into nine subclasses, etc.

A main index card is made for each item in the collection. IBM cards are used. For a collection of bibliographic references, the main index card has the following format:

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0000000001111111112222222222333333333344444444445555555555666666666677777777778
1234567890123456789012345678901234567890123456789012345678901234567890
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00011 2AUTHOR,F AS MUCH OF TITLE AS WILL FIT IN SPACES 20-64/74-CODEN000-0000
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The first four columns give an accession number, ordinarily assigned, i.e. up to 9999 items can be accommodate. The number of authors is given in column 8, the first author in columns 9 and as many more as needed -- if the name goes beyond column 18, it is followed by /. The title begins in column 20 or immediately after the / and is terminated in column 63, followed by / in 64. The last two digits of the year of publication are in columns 65-6, in 68-73 is the 5-letter CODEN designation of the periodical or some special designation, in 73-5 is the volume number, in 77-80 the starting page of the article.

The second and additional authors are placed on card 2; e.g.

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00012  AUTHOR,S           AUTHOR,T           AUTHOR,F
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The main cards are arranged in the appropriate categories, in order of increasing date of publication and then alphabetically by author. By printing the cards one thus obtains a classified index, e.g.:

APPLIED & ENVIROMENTAL CHEMISTRY

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1ERICHSEN JONES,A(JR)/FISH AND RIVER POLLUTION.           /64-/20BK
1BARTLETT,RE PUBLIC HEALTH ENGINEERING-DESIGN IN METRIC /71-/21BA
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By a simple computer program one can get, from the same cards, an index by first and second authors; e.g.

27771	3AXTELL,LM	END RESULTS IN CANCER,REPORT NO.4	/72-/31GD
28712	BACQ,ZM		
28391	1BARTLETT,RE	PUBLIC HEALTH ENGINEERING-DESIGN IN METRIC	/71-/21BA
27772	CUTLER,SJ	MYERS,MH	
28511	1DAVIS,EM	BOD VS COD VS TOC VS TOD.	/71-JAWWA062-0784

(one can, of course, alphabetize all the authors, but that is not very useful).

The system briefly described above is being presented in a more detailed way in a paper that will be submitted for publication.

Moreover, it is planned to utilize the system in an information storage and retrieval system, which will be developed in the future.

REFERENCES

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