Π.27.2
—————
342

NITRATE REMOVAL DURING STORAGE, A REVIEW OF POSSIBLE MECHANISMS AND A SUMMARY OF OBSERVED CHANGES

April 1973

CONTENTS

1. SIGNIFICANCE OF NITRATES

Nitrates are an important part of the food chain and are thus widely used as fertilizers. However, the presence of nitrates in drinking water can produce some very undesirable effects (1)(4).

It was demonstrated as early as 1868 (39) that the addition of amyl nitrate to the blood could result in the formation of methaemoglobinaemia in mice. But it was not until 1945 (38) that Comly showed that nitrates in well waters could be the cause of infantile methaemoglobinaemia, a condition confined to babies under 8 months fed with preparations made from water with a high nitrate concentration. Many authors since then have cited cases of infantile methaemoglobinaemia and it has been shown (6) that, although many factors associated with the disease are not fully understood, in all reported cases there has been a high concentration of nitrate-nitrogen in the drinking water. Nitrate is reduced by bacterial action in the intestine to nitrite and this combines with the haemoglobin in the blood to form methaemoglobin which reduces the oxygen carrying capacity of the blood (7).

In recent years (1)(5)(6) there has been growing concern about the carcinogenic properties of nitrosamines. Nitrate, having been reduced to nitrite may combine with primary and secondary amines present in food to form nitrosamines. Nitrates are often added to certain foods for colouring and preservation, thus increasing the quantity of nitrate consumed. Should nitrosamine carcinogenicity prove to be a real hazard then it may be necessary to reduce nitrate concentrations in water in certain areas.

2. SOURCES OF NITRATE

Nitrate deposits are widely distributed in all parts of the world as potassium, calcium and sodium salts (2).

Nitrogen compounds are produced naturally from atmospheric nitrogen and from decaying plants and animals by bacteria and algae.

Under the influence and action of living organisms ammonia may be produced from protein, urea, amino acids and other nitrogenous compounds. Ammonia may be oxidized to nitrate under aerobic, normally slightly alkaline conditions, and highest rates occur at 30 to 35°C (3).

In order to supplement naturally produced nitrates farmers use considerable quantities of nitrogenous fertilizers, some of which find their way into the river systems. A further source of nitrogen in water is from sewage. A schematic diagram of the nitrogen cycle is shown in Fig. 1 (66).

3. NITRATE CONCENTRATION STANDARDS AND MEASUREMENTS

These dangers of high nitrate concentration in drinking water naturally cause concern and recommended standards have been set (8)(9)(10)(11)(12). It is generally agreed that drinking water should contain less than 11.3 mg NO_3 - N/litre (50 mg NO_3/litre), but 11.3 to 22.6 mg NO_3 - N/litre (50 to 100 mg NO_3/litre) is acceptable and anything greater than 22.6 mg NO_3 - N/litre is not recommended.

It is also recommended that physicians in an area where the nitrate is within the acceptable range between 11.3 and 22.6 mg NO_3 N/litre should be warned of the possible hazard. More recently the World Health Organisation in the International Standards for Drinking Water (9) have found it necessary to reduce the recommended level to not greater than 10.8 mg NO_3 N/litre (45 mg NO_3/litre). In this recent edition of International Standards the possible problem of the formation of nitrosamines in the body is discussed. It is stated that it may be necessary to reduce nitrates in water because of this factor if it is shown that their presence represents a significant health hazard. In a paper by Martin (6) it is recommended wherever possible, if it is impractical to reduce the nitrate level below the recommended standard, to warn local doctors, nurses and health visitors so that they are aware of the possible health hazards and parents should be warned against prolonged boiling of water used to make up infant feeds since this can concentrate the nitrate.

There are four recommended methods for the estimation of nitrates in water.

    (a)   the phenol disulphonic acid method (41)

    (b)   the brucine method (41)

    (c)   reduction with a zinc-copper couple followed by Nesslerization, either directly or after distillation (42)

    (d)   the salycylic acid method (43).

4. CHEMICAL AND PHYSICAL METHODS OF NITRATE REMOVAL

There are two principal methods for chemical or physical removal of nitrogen from water. Namely ammonia stripping (13)(14), and selective ion exchange (13)(14).

4.1. Ammonia stripping

In order to achieve significant nitrate removal by this method the nitrate must be first reduced to ammonia or the ammonium ion. Ammonia stripping operates on the principle of converting the ammonium ions to ammonia gas and then stripping off the ammonia gas by contacting the water with ammonia-free air. Above pH 11 most of the ammonium ions will be converted to ammonia gas. At air temperatures below $0^{\circ}C$, freezing of water occurs at the air inlet, making the process inoperable.

In sewage works much of the nitrogen is present as urea and attempts to remove the nitrogen by ammonia stripping are frustrated because the high pH inhibits urea hydrolysis to ammonia. At a later stage of the process at lower pH hydrolysis to ammonia may take place, leaving an inorganic nitrogen source which could produce the undesirable nitrate ions.

Because of the need to reduce nitrate to ammonia before stripping, the relatively low concentrations of ammonia, the cost of raising the pH to 11 and the restrictions on operating temperature this process is not an attractive one for nitrate removal.

4.2. Ion exchange

The present ion exchange resins can be used effectively for nitrate removal provided the hardness, sulphate and monovalent anion concentrations in the water are low. High concentrations of these anions will reduce the effectiveness of the resin for nitrate removal until such time as a resin has been developed that has a high selectivity for nitrate over other anions in water.

Continuous ion exchange (67) is being used to remove nitrate from a ground water. The process is able to reduce a nitrate concentration of

50 mg NO_3 - N/litre to less than 10 mg NO_3 - N/litre, but no indication is given of how the concentrated nitrate is disposed of after removal by brine from the ion exchange unit.

5. BIOLOGICAL PROCESSES

There are three major processes under this title.

(a) Bacterial Assimilation

(b) Harvesting of algae

(c) Bacterial nitrification - denitrification

One, two or all three of these processes could be taking place in a biologically active system used for removing nitrogen from water.

5.1. General mechanisms

There are two distinct ways in which micro organisms utilize nitrate (40)(45) assimilation and dissimilation.

Nitrate assimilation is the overall process whereby nitrate - N is reduced to ammonia with the subsequent formation of nitrogenous cell constituents. Nitrate dissimilation is an oxidative process in which organisms use nitrate as a source of oxygen; nitrogen does not become incorporated into the cell constituents. In nitrate dissimilation the end product of reduction depends on the organism; it can be nitrite, ammonia, nitrous oxide or nitrogen. If either of the latter two are formed the process is called denitrification. Since the requirement of a cell is greater for oxygen than for nitrogen, the former being used as a source of energy, dissimilation and denitrification proceed at a faster rate than does assimilation.

An effective process for nitrate removal must involve the oxidation of all inorganic nitrogen compounds to nitrate before reduction takes place, because any unoxidized inorganic nitrogen compounds remaining will be oxidized to nitrate after denitrification has occurred.

A large number of bacteria and fungi can reduce nitrate for respiration (i.e. dissimilation) but it seems that a much smaller number assimilate nitrate into cell material. Many organisms which denitrify require ammonia or amino acids for cell synthesis.
4.

The presence of a carbon source, or sometimes molecular hydrogen, which can be oxidized is an essential requirement for the reduction of nitrate. Also required in macro quantities are the mineral nutrients sulphate, phosphate, chloride, sodium, potassium, magnesium and calcium and various trace elements are required for successive enzymic steps; metals reported to be necessary (52) are molybdenum, iron, copper and manganese.

Dissimilation normally requires completely anaerobic conditions, whereas nitrate reduction by assimilation can take place under aerobic conditions.

Organisms usually require a period of adaption to nitrate and anaerobic conditions for dissimilation, but this period is not normally required for assimilation.

The process of dissimilation is sensitive to oxygen whereas assimilation is unaffected.

5.2. Effect of pH

The optimum value of pH for the reduction of nitrate depends on the nitrate concentration, age of the culture and the organism concerned (54). The end product and enzyme activity depend on the pH (47)(55)(61); above pH 7 little N_2O was formed while below pH 7 the gas was evolved. NO was evolved between pH 6 and 7 and as much as 20% of the gas evolved at pH 5 was NO.

A series of experiments (44) showed that the optimum pH for nitrification of ammonia to nitrite by <u>Nitrosomonas</u> and oxidation of nitrite to nitrate by <u>Nitrobacter</u> was 8.4.

5.3. Temperature

Several authors (19)(20)(23)(28)(30)(36)(48) have indicated that the rate of nitrate removal by a nitrification-denitrification process is temperature dependent. Dawson and Murphy (23) in their paper on the temperature dependency of biological denitrification showed a fivefold rate increase as the temperature rose from $5^{\circ}C$ to $20^{\circ}C$. They also showed that an acclimatization period was needed during which time about a quarter of the total

nitrate was removed. At $5^{\circ}C$ this period was 8 days and was reduced to 5 hours at $27^{\circ}C$.

5.4. Oxygen level

The presence of oxygen in water may affect the rate of nitrification/ denitrification processes. According to Slechta and Culp (46) in order to accomplish significant removal of inorganic nitrogen all of the nitrogen compounds must be converted to nitrate before denitrification takes place.

Removal of nitrate by assimilation is unaffected by oxygen concentrations; thus algae and plants are able to use nitrate assimilatively in lakes and rivers. The effect of oxygen on dissimilative enzymes does not seem to be quite as clear. However, it seems to be generally agreed (19)(37)(40)(45)(47)(48)(51)(56)(57) (58)(59)(60) that the presence of oxygen does reduce the rate of denitrification and that denitrification may take place because as a result of oxygen gradients some of the bacteria may be at zero oxygen concentration, although the general body of water contains oxygen.

The rate of nitrification, which affects the rate of the nitrification- denitrification process, has been shown to be independent of the ammonia concentration, proportional to the number of nitrifiers present and independent of instantaneous changes in BOD. However changes in the average BOD level can influence the nitrification rate because this affects the concentration of nitrifiers present (44).

As mentioned earlier, molecular oxygen usually inhibits denitrification. In those organisms where nitrate reduction was linked with cytochrome (53), inhibitors of cytochrome, e.g. 2-n-heptyl-4-hydroxyquinoline-N-oxide, would inhibit nitrate reduction.

5.5. Organic carbon requirements

An essential requirement for nitrate dissimilation is a carbon source (47)(52). Much work has been done on this aspect of denitrification to try and determine the type of carbon source required and the optimum quantity of the carbon needed for denitrification.

6.

Many organic carbon sources such as glucose, peptone, malate, lactate, ethanol, acetic acid, acetone, glycerol, raw sewage, methanol and methane have been investigated over a period of time by many authors (15)(22)(24)(25)(26) (27)(28)(29)(31)(33)(36)(37)(46)(47)(48)(63)(64).

The simple organic compounds, such as those mentioned above, have been found to be more effective hydrogen donors for micro-organisms reducing nitrate than more complex carbon compounds.

In general settled domestic sewage was found to be unsuitable as a carbon source for inducing denitrification, it also added BOD and unoxidized nitrogen to the final effluent, which may later be oxidized to nitrate.

McCarty et al (47) and Mulbarger (48) using acetone, acetic acid, ethanol and methanol as hydrogen donors showed that the organic carbon type has a significant effect on the rate of denitrification and resulted in varying accumulations of nitrite as an intermediate. Davies et al (24)(25) using glucose and malate expanded on this idea and showed that not only did denitrification rate depend on the carbon source present, but there was a change in bacterial population with change in carbon source.

Slechta and Culp (46) reported using sugar, ethanol, acetic acid and methanol as the sole carbon source and indicated that methanol was the cheapest material to use. Since then many workers (22)(26)(27)(29)(64) have used methanol as both a hydrogen donor and a carbon source for organism growth. St. Amant and McCarty (29)(47) presented a method of estimating the methanol needed for denitrification and predicted a methanol: nitrate-nitrogen ratio of about 2.5:1. Stensel et al (22) proposed a similar ratio whilst Barth (68) suggested the ratio should be 4:1.

The methanol requirements predicted by St. Amant and McCarty (29)(47) and Stensel et al (22) have been confirmed by work on municipal wastes by Smith and his co-workers (37) and by Du Toit and Davies (28) whose work suggested that the ratio of 4:1 is too high.

Wuhrmann (64) suggests that no organic carbon source need be added to activated sludge because there are sufficient reserves of oxidizable material present to maintain metabolism so that, in anaerobic conditions, the bulk of oxidized nitrogen present in solution will be reduced to gaseous nitrogen given sufficient time. The addition of carbonaceous material may increase the rate of denitrification and Wuhrmann (64) and Bayley (36) found that the predicted methanol:nitrate-nitrogen ratio of 2.5 : 1 (29)(47) was sufficient to fulfill the denitrification requirements.

More recently Davies (63) has shown that methane could be an ideal carbon source in commercial denitrifying units. Being a gas, it would eliminate the need for monitoring the nitrate in the water in order to avoid the addition of excess organic carbon which would, in itself, constitute pollution. Denitrification has been achieved with methane as the sole electron donor in pure culture and mixed cultures derived from settled sewage. Methods of preparation of denitrifying bacteria that can use methane as an organic carbon source are described. Bacteria adapted to methane were used and tests were also carried out on bacteria adapted to methanol to see if they could use methane as a hydrogen donor in nitrate respiration.

Strong growth occurred in most of the bacteria isolated from the unit receiving methane, the gas evolved was shown to be nitrogen.

Several bacteria isolated from the methanol enrichment culture were found to be capable of growth and denitrification with methane as sole carbon source. This made it apparent that no specialized forms of bacteria are needed to use methane as a carbon source for denitrification.

All isolates taken from both denitrifying enrichments which showed the ability to denitrify using methane as hydrogen donor were capable of using all the other carbon compounds tested. Nitrogen was produced with methanol, ethanol, malate or lactate as sole carbon source. None of the isolates, however, was found to utilize molecular hydrogen for denitrification.

8.

A packed column laboratory scale denitrifying unit maintained at 30°C achieved 50 percent denitrification with methane as sole organic carbon source. If a system can be devised to ensure adequate methane in solution, then it should be possible to appreciably reduce the cost of obtaining good denitrification rates, making it a possible alternative to other methods of nitrogen removal from water. However, it must be recognized that methane is already used as a cheap source of energy at sewage works and the overall economics would need careful consideration.

5.6. Trace elements

Some metal chelating agents inhibited the formation of nitrate reductase. The most potent of these were potassium cyanide and 4-methyl-1:2-dimercaptobenzene. Chlorate, copper and p-chloromercuri-benzoate also acted as inhibitors (61)(62).

6. STUDIES ON RIVERS AND WASTE WATERS

From the above work it would be expected that nitrite, elementary nitrogen and ammonia would be produced from nitrate at low dissolved-oxygen concentrations (< 0.5 mg/litre) in rivers, sewage, etc. provided the necessary enzymes have been induced previously under anaerobic conditions. A very few organisms have been reported to denitrify under highly aerobic conditions.

There are practical difficulties in demonstrating aerobic denitrification in rivers in situ, largely because penetration of oxygen into mud deposits, from the overlying liquid is limited by diffusion with the result that oxygen-concentration gradients within the mud are formed culminating in anaerobic conditions some distance below the surface. For example, in a study of a $1\frac{1}{2}$ mile stretch of the R. Lark below a sewage-effluent out fall (69) it was found that in a given body of water the concentration of nitrate fell from 3.3 to 1.6 mg N/litre and that of nitrite rose from 0.2 to 0.6 mg/litre while the concentration of ammonia stayed constant. Since the concentration of dissolved oxygen at no time fell below 1.3 mg/litre, and was usually much higher, it was postulated that anaerobic denitrification had occurred in the mud. No organic nitrogen concentrations in the water were determined so aerobic assimilation cannot be ruled out.

In laboratory experiments (70) on river water in open 5 litre beakers the aqueous phase was maintained at about saturation with regard to oxygen; mud was omitted or present as a stagnant bottom layer. Only in the latter case was nitrate reduced. The rate of nitrate removal was only about 10% of that reported for the R. Lark (69) i.e. 0.04 mg N/litre/hr. In similar experiments with Thames mud (69) the average rates over 30 days were even lower, ranging from 0.01 to 0.005 mg N/litre/hr.

It has been established (71) that in the Thames Estuary nitrification and denitrification are important processes; in the anaerobic regions about 25 percent of the oxygen necessary for carbonaceous oxidation was derived from nitrate some of which was formed from ammonia in the aerobic parts of the estuary and in tributaries. There are considerable difficulties in achieving a nitrogen balance for any river and because no organic nitrogen determinations were made a complete balance cannot be made from data of the Thames Estuary.

Hermann (17) reported significant nitrate removal in waste stabilization ponds. Waste waters with up to 110 mg N/litre were amenable to treatment but the efficiency of the biological system to reduce nitrates declined for loadings greater than 70 mg N/litre from about 80% removal at 70 mg N/litre to less than 50% removal at 120 mg N/litre. Denitrification occurred regardless of the BOD:nitrogen ratio, thus supporting the theory that in the stabilization pond, bacteria and algae are mainly responsible for the oxidation and reduction reactions that occur, and that chemically combined oxygen may enter into the satisfaction of BOD.

McKinney et al (30)(32) carried out laboratory experiments to determine fundamental parameters such as a light-dark cycle, detention time, light intensity and biomass concentration in an algal-bacterial system for nutrient removal using artificial light. The results indicated that a light-dark relationship of 3.3 minutes in the light and 2.4 minutes in the dark and a 10 hour retention time made the process effective. With a mixed liquor suspended solids (MLSS) concentration of 1000-1400 mg/litre and a light

intensity of 400 ft-c, 92 percent removal of nitrogen and 97 percent removal of BOD was achieved.

The work mentioned so far has been carried out for sewage treatment or in controlled laboratory experiments. It is felt that the results from such work have a direct bearing on the understanding of the mechanism involved for the removal of nitrate from stored waters, especially as many of these waters contain sewage effluent.

As stated earlier (71) it is extremely difficult in achieving a complete nitrogen balance and consequently difficult to establish an exact mechanism for nitrate removal from rivers and stored waters.

7. NITRATE CHANGES DURING STORAGE

Below are presented observed results and some suggestions of the possible mechanism of removal for stored waters from the R. Rhine (Berenplaat) (19), R. Lee (Chingford Mill) (35), R. Thames (Laleham) (35), R. Thames (Farmoor) (65), R. Chelmer (Hanningfield) (50), R. Tame (Lea Marston) (16), R. Tame (Elford) (16), and R. Trent (Colwick (18).

As reported by Ferdov and Sergava (51) it was found (19) that the rate of nitrification was independent of ammonia concentration. A stoichiometric increase in nitrate concentration by oxidation of ammonium ions was not observed in any of the stored waters studied (16)(18)(19)(35)(50)(65). In fact, in general, there was a decrease in nitrate concentration. At the Berenplaat reservoir (19), Chingford Mill (35) and Laleham (35) it was observed that during the summer and autumn periods the removal was greater than during the spring and winter periods. No seasonal variations were noted in the other plants.

The seasonal differences of nitrate removal may be due to temperature changes. Windle Taylor (35) suggested that as well as being caused by microbiological action, the reduction could be due to algal growth, the nitrate nitrogen being used in particular by the blue-green algae. However, at the Berenplaat reservoir (19), Farmoor (65), Colwick (18) and Hanningfield (50)

there was no correlation between maximum algal counts and nitrogen loss indicating denitrification to nitrogen gas must be partly responsible for the removal.

At Chingford Mills (35) the spring/winter nitrate reduction was about 26 percent and that of the summer/autumn period was about 40 percent whilst at Laleham they were 15 and 38 percent respectively.

Sedimentation followed by five day storage of river Tame water (16) achieved 6.5 percent nitrate reduction at Elford and about 10 percent reduction at Lea Marston.

At Hanningfield reservoir (50) sewage effluent is discharged into the reservoir, but its contribution to the nitrate concentration is only small compared with land drainage and run off during the winter months. The reservoir has a 270 day storage and the average nitrate-nitrogen concentration of the water entering the reservoir was 12 mg nitrate -N/litre. However, the average concentration in the reservoir was only 4.3 mg/litre and in fact it fell as low as 2 mg/litre. Up to 83 percent removal was achieved by 270 day storage without any abnormal algal growth. Others have observed nitrate removal without excessive algal growth at Colne Valley Water Board and Birmingham CBC Water Department (50).

At Farmoor, Thames water is impounded for a nominal 160 days and approximately 50 percent of the nitrate is removed. Over a four year period from 1968 to 1971 the mean nitrate concentration flowing into the reservoir was 6.4 mg Nitrate-N/litre and was reduced to 2.7 mg/litre. Weekly samples of nitrate were taken and there appeared to be no particular pattern of algal development with nitrate removal.

A more detailed survey has been carried out on the river Trent at Colwick (18) to study the effect of 21 day storage on water quality. The raw water passed through three tanks in series each with a nominal retention time of 7 days (see Fig. 2) and chemical and biological analyses were made, see Table 1. It can be seen from Fig. 3 (18) that the first and second weeks of

storage achieve a high proportion of nitrate removal and in fact the third weeks storage has only a marginal effect.

During September and October 1970 one weeks storage removed 50 percent of the nitrate, followed by no further denitrification during the second and third weeks. After this period there was a general decrease in nitrate concentrations in all three tanks even though the raw water level remained almost constant. By August 1971 100% removal of nitrate was being effected after two weeks storage.

In view of the high nitrate removal after only two weeks storage it was decided, for the present phase of work, to modify the plant (see Fig. 4) to two parallel streams, each with 14 days retention. The chemical-biological analysis was also modified as indicated in Table 1.

The nitrate removal figures for one stream are presented in Fig. 5; the results for the second stream are almost identical.

The same degree of nitrate removal has not yet been achieved by this system. The raw water nitrate concentrations have remained reasonably steady at 8-9 mg N/litre. During August/September 1972 there was about 40 percent removal of nitrate but until the last couple of weeks there was either no change or a slight increase in nitrate concentrations.

The last three weeks of March have shown an improvement in the situation with some nitrate removal as indicated in Fig. 5. Accompanying this removal is a high algal count.

However, the reasons for these changes of fortune from one system to the next and even with time in the same system are not clear, but they do emphasize the fact that the whole mechanism of nitrate removal is a very complex process dependent on many independently and dependently operating parameters.

The mechanism for nitrate removal is still not solved but many of the factors influencing the removal of nitrate have been identified. Further work at Colwick will involve changing variables on one stream in an attempt to vary nitrate removal. Initially local anaerobic conditions will be encouraged by methanol dosing and later, if nitrate removal proceeds, covering of one tank to exclude light will be tried in an attempt to assess the contribution algae make to this process.

CONCLUSIONS

Changes of nitrate concentration in stored waters can take place. The mechanism and factors influencing rate and direction of reactions are interrelated and complex.

Nitrogen assimilation by bacteria and algae and bacterial nitrification-denitrification are the principal stages in nitrate removal and their performance is dependent on pH, temperature, oxygen concentration, an organic carbon source, and the presence or absence of various trace elements and mineral nutrients.

In a reservoir conditons are mainly aerobic with local anaerobic sites in the top layer and an anaerobic bottom layer.

Removal of both oxidized and unoxidized inorganic nitrogen compounds in the top layer is mainly by assimilation. However, in the local anaerobic sites in the top layer and in the anaerobic bottom layer nitrate removal is achieved by dissimilation.

There is evidence of ammonia production in the bottom layer, possibly from decaying organisms. This ammonia may be oxidized to nitrate when it comes into contact with the aerobic top layer.

14.

The complexity of the mechanism of nitrate removal in stored waters is demonstrated by the work carried out at Colwick on Trent water. For no apparent reason there are changes in trends and sudden rises and falls in nitrate concentration.

Though many of the parameters influencing nitrate removal have been identified the overall mechanism of removal had not yet been established.

To what degree nitrate uptake by algae, bacterial assimilation or bacterial dissimilation is the predominant factor is not known. Certainly each has a role to play in nitrate removal and the importance of that role will be influenced by the factors mentioned above, the bacteria and algae present and the residence time of the water in the system.

In order to be able to control and use these mechanisms for an efficient reliable method of removal of nitrate from water more research will have to be carried out.

REFERENCES

1. BWA COMMITTEE ON WATER QUALITY AND TREATMENT — "Nitrates in water supplies" London, The Association (1971).

2. TASK GROUP REPORT — "Sources of nitrogen and phosphorus in water supplies" JAWWA, 59, 344 (March 1967).

3. STARR NICHOLS, M. — "Nitrates in the environment" JAWWA, 57, 1319 (Oct. 1965).

4. WELSH, G.B., and THOMAS, J.F. — "Significance of chemical limits in USPHS drinking water standards" JAWWA, 52, 289 (March 1960).

5. WOLFF, I.A., and WASSERMAN, A.E. — "Nitrates, Nitrites and Nitrosamines" Science, 177, 15 (1972).

6. MARTIN, A.E. — "Medical considerations in the abstraction of potable waters from polluted sources" Journal of the Society for Water Treatment and Examination, 21 (3), 202 (1972).

7. LENAIN, A.F. — "The impact of nitrates on water use" JAWWA, 59, 1049 (Aug 1967).

8. BURDEN, E.H.W.J. — "The toxicology of nitrates and nitrites with particular reference to the potability of water supplies - A review" Analyst (Brit) 86, 429 (1961).

9. WORLD HEALTH ORGANIZATION — "International standards for drinking water" Third edition. Geneva (1971).

10. WORLD HEALTH ORGANIZATION — "European standards for drinking water" Second edition. Copenhagen (1970).

11. US PUBLIC HEALTH SERVICE — "Drinking water standards" Publ. No. 956 US Government printing office. Washington DC (1962).

12. KAPANDZE, S.K. — "Maximum permissible concentrations of nitrates in water" Gigiena I Sari (USSR) 26, 7 (1971).

13. SHINDALA, A. — "Nitrogen and phosphorus removal from waste waters" Water and Sewage Works 119, 60 (July 1972).

16.

14. CULP, R. L., and
    CULP, G. L.

"Advanced wastewater treatment"
van Nostrand Reinhold Company.

15. MATSCHÉ, N.

"The elimination of nitrogen in the
treatment plant of Vienna-Blumental"
Water Research, 6, 485 (Apr/May 1972).

16. LESTER, W. F.,
    WOODWARD, G. M., and
    RAVEN, T. W.

"The effect and cost of river purification
lakes"
The Institute of Water Pollution Control,
74, (Apr 1971).

17. HERMANN, E. R.

"Stabilization ponds as a nitrate reducing
reactor"
Proc. Amer. Soc. Civ. Engr, 88, SA5,
1 (1962).

18. WATER RESEARCH
    ASSOCIATION

"Trent Economic Model"
Trent Final Report.

19. ROOK, J. J., and
    OSKAM, G.

"Biological and chemical aspects of
Rhine water in the Berenplaat Reservoir"
JAWWA, 62, 249 (Apr 1970).

20. LANCE, J. C.

"Nitrogen removal by soil mechanisms"
Journal WPCF, 44, 1352 (July 1972).

21. MOORE, S. F., and
    SCHROEDER, E. D.

"The effect of nitrate feed rate on
denitrification"
Water Research, 5, 445 (July 1971).

22. STENSEL, H. D.,
    LAWRENCE, A. W., and
    LOEHR, R. C.

"Equations relating methanol, nitrate
removal and dissolved oxygen"
Water and Wastes Engineering 9, 16
(March 1972).

23. DAWSON, R. N., and
    MURPHY, K. L.

"Temperature dependency of biological
denitrification"
Water Research, 6, 71 (Jan 1972).

24. DAVIES, T. R., and
    TOERIEN, D. F.

"Population description of a denitrifying
microbial system"
Water Research, 5, 553 (Aug 1971).

25. DAVIES, T. R., DuTOIT, P. J.,
    and TOERIEN, D. F.

"Selection patterns in a denitrifying
population"
Water Research, 5, 353 (Aug 1971).

26. BARTH, E. F.

"Design of treatment facilities for the
control of nitrogenous materials"
Water Research, 6, 481 (Apr/May 1972).

27. MULBARGER, M.C.,
    PAGE, G.L.(Jr).,
    YATES, C.W.(Jr)., and
    SHARP, N.C.

    "Manassas, Va., adds nutrient removal
    to waste treatment"
    Water and Wastes Engineering, 6, 46
    (Apr 1969).

28. DuTOIT, P.J., and
    DAVIES, T.R.

    "Denitrification studies with laboratory
    scale continuous-flow units"
    Water Research, 7, 489 (March 1973).

29. AMANT, P.P.St., and
    McCARTY, P.L.

    "Treatment of high nitrate waters"
    JAWWA, 61, 659 (Dec 1969).

30. McGRIFF, E.C. (Jr)., and
    McKINNEY, R.E.

    "The removal of nutrients and organics
    by activated algae"
    Water Research 6, 1155 (Oct 1972).

31. HEMENS, J., MASON, M.H.,and
    STANDER, G.J.

    "Nutrient removal from sewage effluents
    by algal activity"
    Nat. inst. for Water Research WAT 32 p.12.
    1967/68 Ann Rep (Pretoria SA 1968).

32. McKINNEY, R.E., McGRIFF,E.C.
    SHERWOOD, R.J.,
    WAHBEH, V.N.,and
    NEWPORT, D.W.

    "Ahead: Activated algae"
    Water and Waste Engineering, 5, 51
    (Sept 1971).

33. LUDZEACK, F.J., and
    ETTINGER, M.B.

    "Controlling operation to minimize
    activated sludge effluent nitrogen"
    Journal WPCF 34, 920 (Sept 1962).

34. ANON.

    "Phosphate/nitrate removal where
    effluent polishing is put to the test"
    Water and Wastes Engineering 7, 49
    (Nov 1970).

35. WINDLE TAYLOR, E.

    Metropolitan Water Board
    38th Ann Rep. (1960).

36. BAYLEY, R.W.

    "Nitrogen and phosphorus removal:
    Methods and costs"
    Water Treatment and Examination, 19,
    294 (March 1970).

37. SMITH, J.M., MASSE, A.N.,
    FEIGE, W.A., and
    KAMPLACHE, L.J.

    "Nitrogen removal from municipal
    waste water by columnar denitrification"
    Eng. Sci and Tech 6, 261 (March 1972).

38. COMLY, H.H.

    "Cyanosis in infants caused by nitrates
    in well water"
    J. Amer. Med. Ass. 129, 112 (1945).

39. GAMGEE, A.

    "Researches on the blood: On the action
    of nitrites on the blood"
    Phil Trans 158, 589 (1868).

40. PAINTER, H.A.

"A review of literature on inorganic nitrogen metabolism in microorganisms" Water Research, $\underline{4}$, 393 (1970).

41. APHA., AWWA., WPCF.

"Standard methods for the examination of water and wastewater" 12th ed. (1965) N.Y. APHA.

42. IWE., RIC., SAC., SWT & E.

"Approved methods for physical and chemical analysis of water" 3rd ed. (1960), London, Institution of Water Engineers.

43. GESELLSCHAFT DEUTSCHERCHEMIKER; FACHGRUPPE WASSERCHEMIE

"German standard methods for water, waste water and sludge analysis; physical, chemical and biological analysis!' Weinheim/Bergstrasse, Velag Chemie GMBH (1960).

44. WILD, H.E.(Jr)., SAWYER,C.N., and McMAHON, T.C.

"Factors involving nitrification kinetics" Journal WPCF, $\underline{43}$, 1845 (Sept 1971).

45. FEWSON, C.A., and NICHOLAS, D.J.D.

"Utilization of nitrate by microorganisms" Nature, $\underline{190}$, 2 (1961).

46. SLECHTA, A.F., and CULP, G.L.

"Water reclamation studies of South Tahoe public utilities district" Journal WPCF, $\underline{39}$, 787 (1967).

47. McCARTY, P.L., BECK, L., and AMANT, P.St.

"Biological denitrification of waste water by addition of organic materials" 24th Purdue Conference (1969).

48. MULBARGER, M.C.

"Modifications of the activated sludge process for nitrification and denitrification" Amer. Conf. WPCF, Boston, Mass. (1970).

49. MOORE, S.F., and SCHROEDER, E.D.

"An investigation on the effects of residence time on bacterial denitrification" Water Research, $\underline{4}$, 685 (1970).

50. SLACK, J.G.

"Water reclamation from sewage effluent: experimental studies in Essex" Water Treatment and Examination $\underline{21}$, 239 (1972).

51. FEDEROV, M.W., and SERGAVA, R.V.

"The effect of oxidation-reduction conditions of the medium on the rate of nitrate reduction by nitrifying bacteria" Journal WPCF, $\underline{32}$, 110 (1960).

52. NICHOLAS, D.J.D.

"The metabolism of inorganic nitrogen and its compounds in microorganisms" Biol. Rev. $\underline{38}$, 530 (1963).

53. NASON, A.   "Enzymatic pathways of nitrate, nitrite, and hydroxylamine metabolisms" Bact. Rev. $\underline{26}$, 16 (1962).

54. DELWICHE, C.C.   "Denitrification" Symp. on inorg. nitrogen met. p. 233. (Ed. W.D. McElroy & B.Glass). John Hopkins Pass, Baltimore (1956).

55. WIJLER, J., and DELWICHE, C.C.   "Investigations of the denitrifying process in soil" Pl. Soils $\underline{5}$, 155 (1954).

56. SACKS, L.E., and BAKER, H.A.   "Influence of oxygen on nitrate and nitrite reduction" J. Bact. $\underline{58}$, 11 (1949).

57. MARSHALL, R.O., DISHBURGER, H.J., MacVICAR, R., and HALLMARK, G.D.   "Studies on the effect of aeration on nitrate reduction by pseudomonas species using N^{15}" J. Bact. $\underline{66}$, 254 (1953).

58. SKERMAN, V.B.D., and MacCRAE, J.C.   "The influence of oxygen on the reduction of nitrate by adapted cells of pseudomonas denitrificans" Can J. Microbiol $\underline{3}$, 215 (1957).

59. SKERMAN, V.B.D., and MacCRAE, I.C.   "The influence of oxygen availability on the degree of nitrate reduction by pseudomonas denitrificans" Can. J. Microbiol $\underline{3}$, 505 (1957).

60. JANNACH, H.W.   "Denitrification as influenced by Photosynthetic oxygen production" J. Gen. Microbiol $\underline{23}$, 55 (1960).

61. FEWSON, C.A. and NICHOLAS, D.J.D.   "Nitrate reductase from pseudomonas aeruginosa" Biochim, biophys Acta $\underline{49}$, 335 (1961).

62. WALKER, C.G., and NICHOLAS, D.J.D.   "Nitrate reductase from pseudomonas aeruginosa" Biochim, biophys Acta $\underline{49}$, 350 (1961).

63. DAVIES, T.R.   "Isolation of bacteria capable of utilizing methane as a hydrogen donor in the process of denitrification" Water Research, $\underline{7}$, 575 (Apr 1973).

64. WUHRMANN, K.   "Objectives, technology, and results of nitrogen and phosphorus removal processes' Advances in Water Quality Improvement. E.F.Glovna: W.W.Eckenfelder. Uni. Tex. Press. Austin and London (1968) p. 21.

65. WATER RESEARCH ASSOCIATION

Unpublished data.

66. McCARTY, P.L.

"Biological processes for nitrogen removal-theory and application"
Proc. 12th San. Eng. Conf., Ill. Uni. Dept. Civ. Eng. (1970) p. 136.

67. HOLZMACHER, R.G.

"Nitrate removal from a ground water supply"
Water and Sewage Works 118, 210 (July 1971).

68. BARTH, E.F., BRENNER, R.C., and LEWIS, R.F.

"Chemical-biological control of nitrogen and phosphorus in waste water effluent"
Journal WPCF 40, 2040 (1968).

69. DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

Water Pollution Research (1962), HMSO. London.

70. EDWARDS, R.W., and ROLLEY, H.L.J.

"Oxygen consumption of river muds"
J. Ecol. 53, 1 (1963).

71. DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

"Effects of polluting discharges on the Thames Estuary"
Water Pollution Research TP. NO. 11 (1964) HMSO. London.

?

TABLE 1

BIOLOGICAL AND CHEMICAL ANALYSIS

	FEED WATER	ORIGINAL STORED SYSTEM	MODIFIED STORED SYSTEM
Shaken turbidity	C	W	-
Colour	D	W	-
Total organic carbon (filtered)	2W	W	-
P. V. (filtered)	W^+	W	-
pH	C	W	-
Ammonia	D	W	W
Nitrite	W^+	W	W
Nitrate	W^+	W	W
Alkalinity	D	W	-
Total hardness	D	W	-
Dissolved oxygen	W	W	-
Temperature	C	W	-
Chlorophyll	W^+	W	-
Coliforms	W^+	W	W
E. Coli	W^+	W	W
Plate count	W^+	W	W
Inlet flow	W^+	W	W
Anionic detergents	W^+	M	-
Non-ionic detergents	W^+	M	-
Monohydric phenols	W^+	M	-
Threshold odour number	W^+	M	-
Electrical conductivity	D	M	-
Calcium hardness	D	M	-
Chloride	W^+	M	-
Sulphate	W^+	M	-
Orthophosphate	W^+	M	-
Total iron	$W^\times$	M	-
Total manganese	$W^\times$	M	-
Total cadmium	$W^\times$	M	-
Total chromium	$W^\times$	M	-
Total nickel	$W^\times$	M	-
Total copper	$W^\times$	M	-
Total lead	$W^\times$	M	-
Total zinc	$W^\times$	M	-
Cyanide	$W^\times$	M	-
Algal count	W	M	W
Total nitrogen	W	-	W
Denitrifying bacteria	W	-	W

C = continuously monitored
D = measured daily
W = measured weekly
M = measured monthly
+ = river water
× = river water, filtered 0.45 μ membrane.
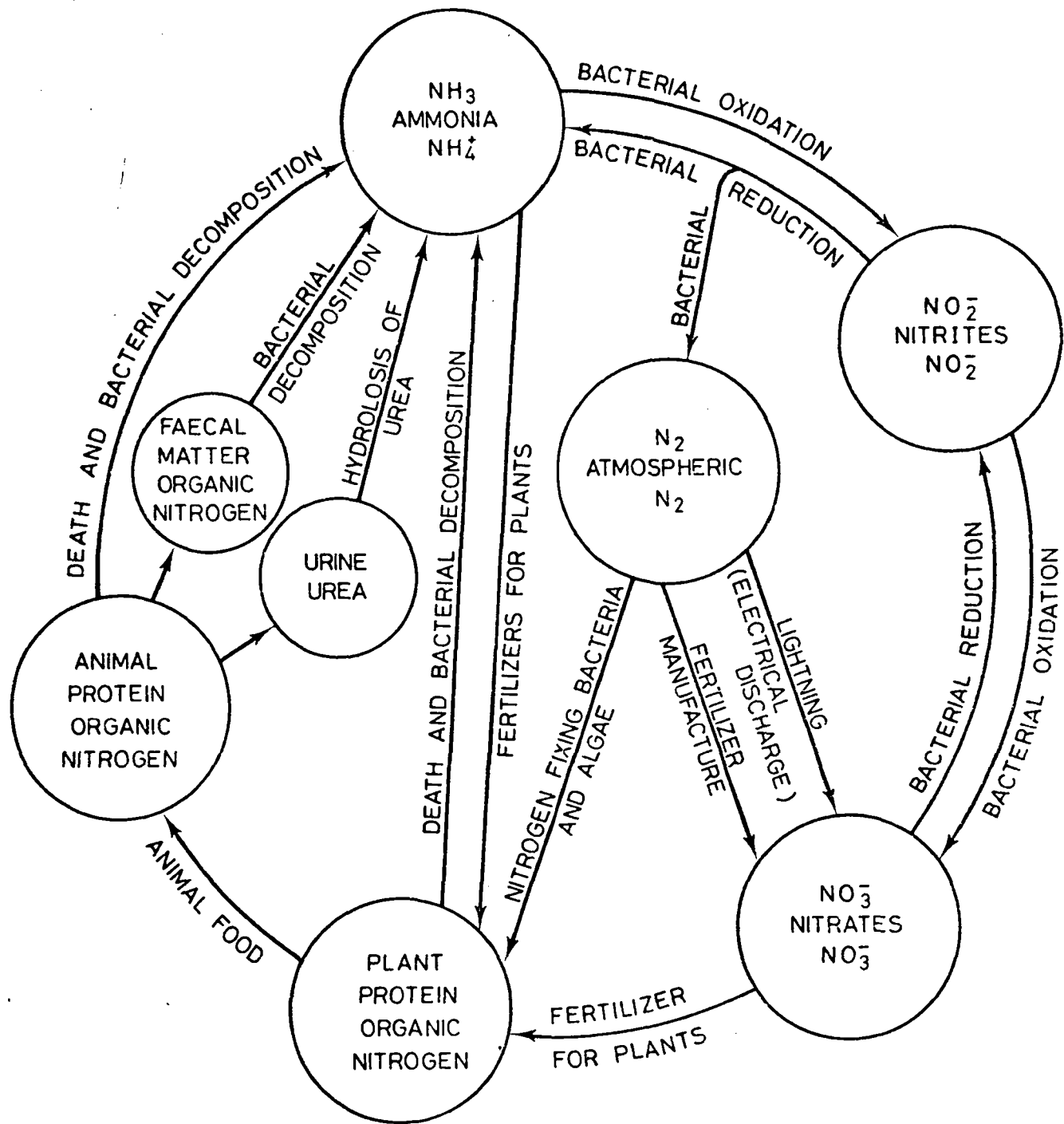2W = measured twice weekly.
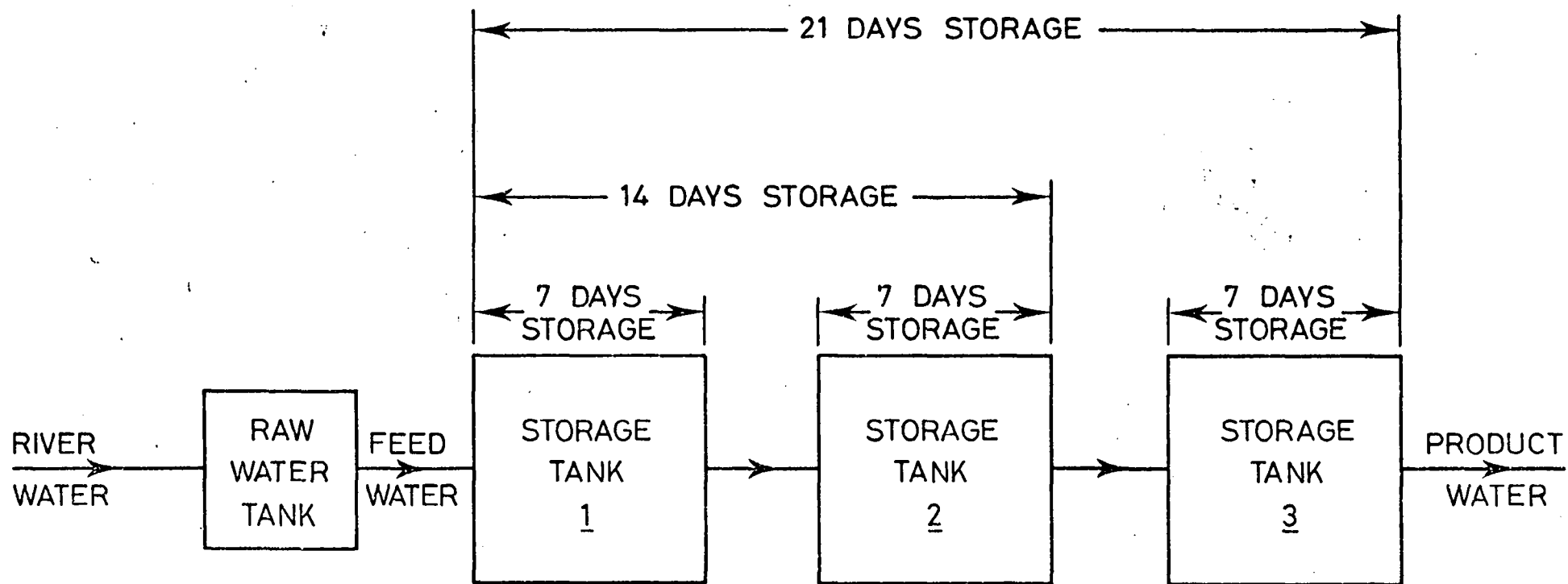
FIG. 1. THE NITROGEN CYCLE

FIG.2. ORIGINAL STORAGE SYSTEM FOR TRENT WATER AT COLWICK
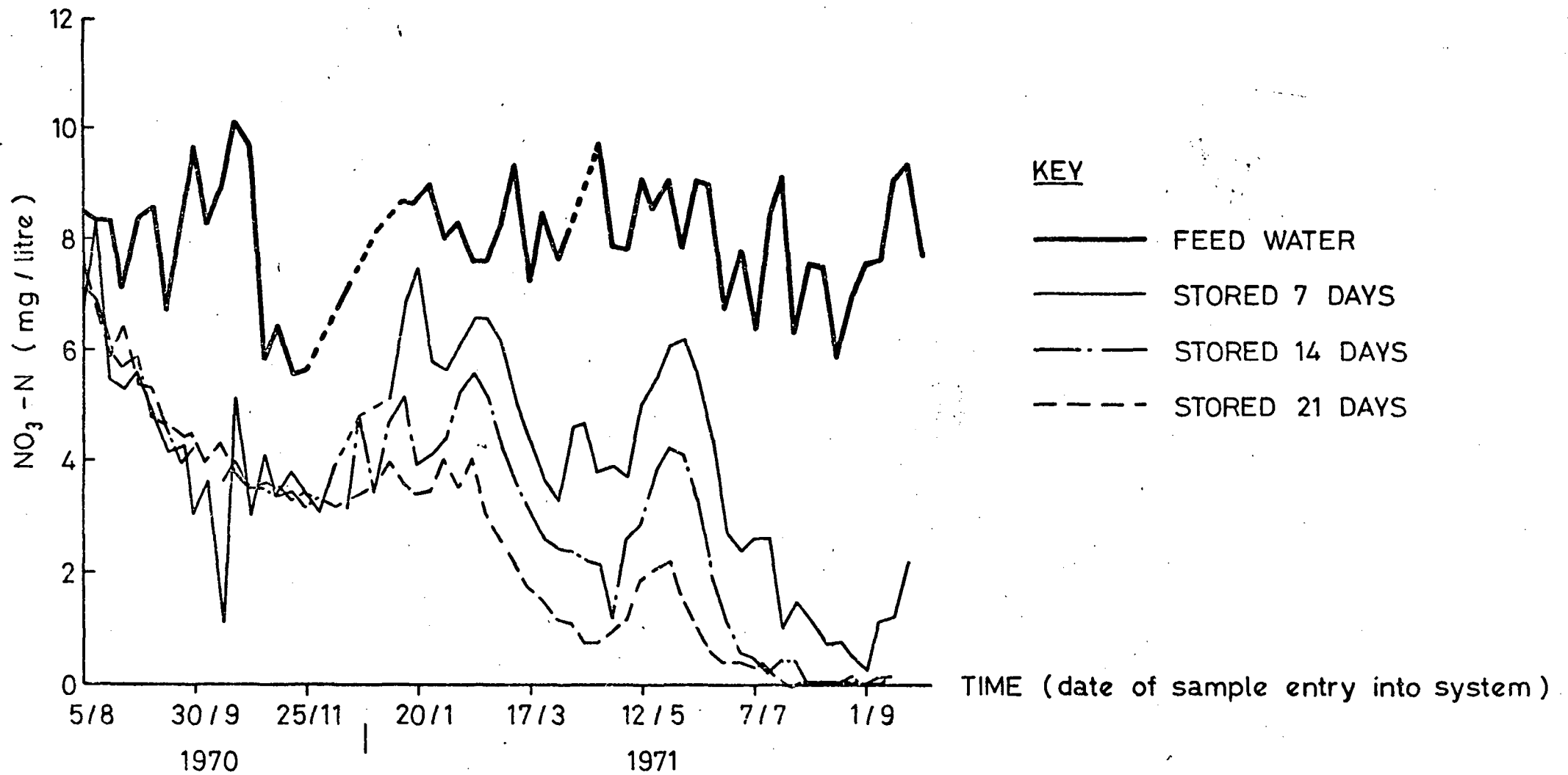
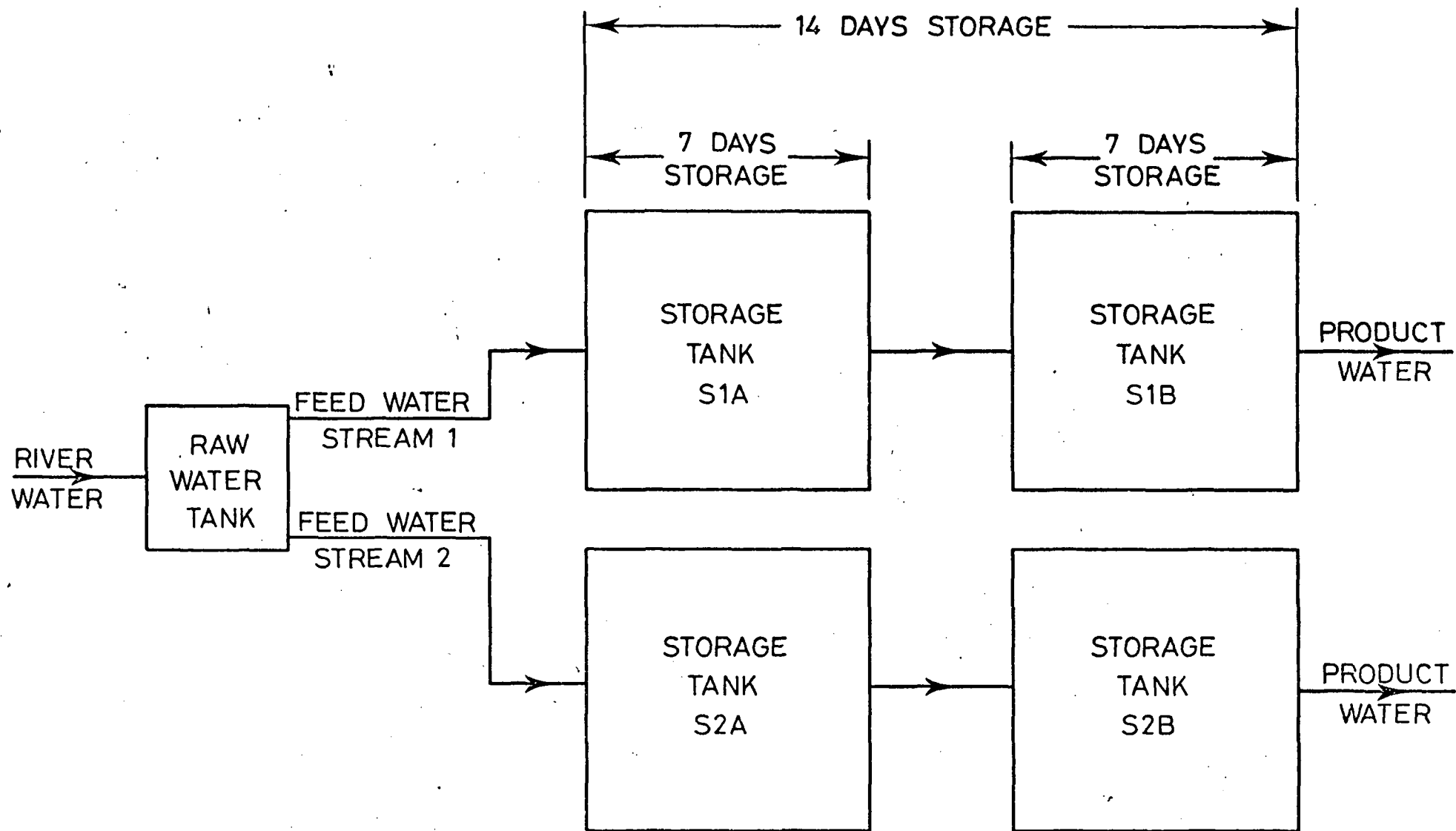FIG.3. EFFECT OF STORAGE ON NITRATE - ORIGINAL PLANT

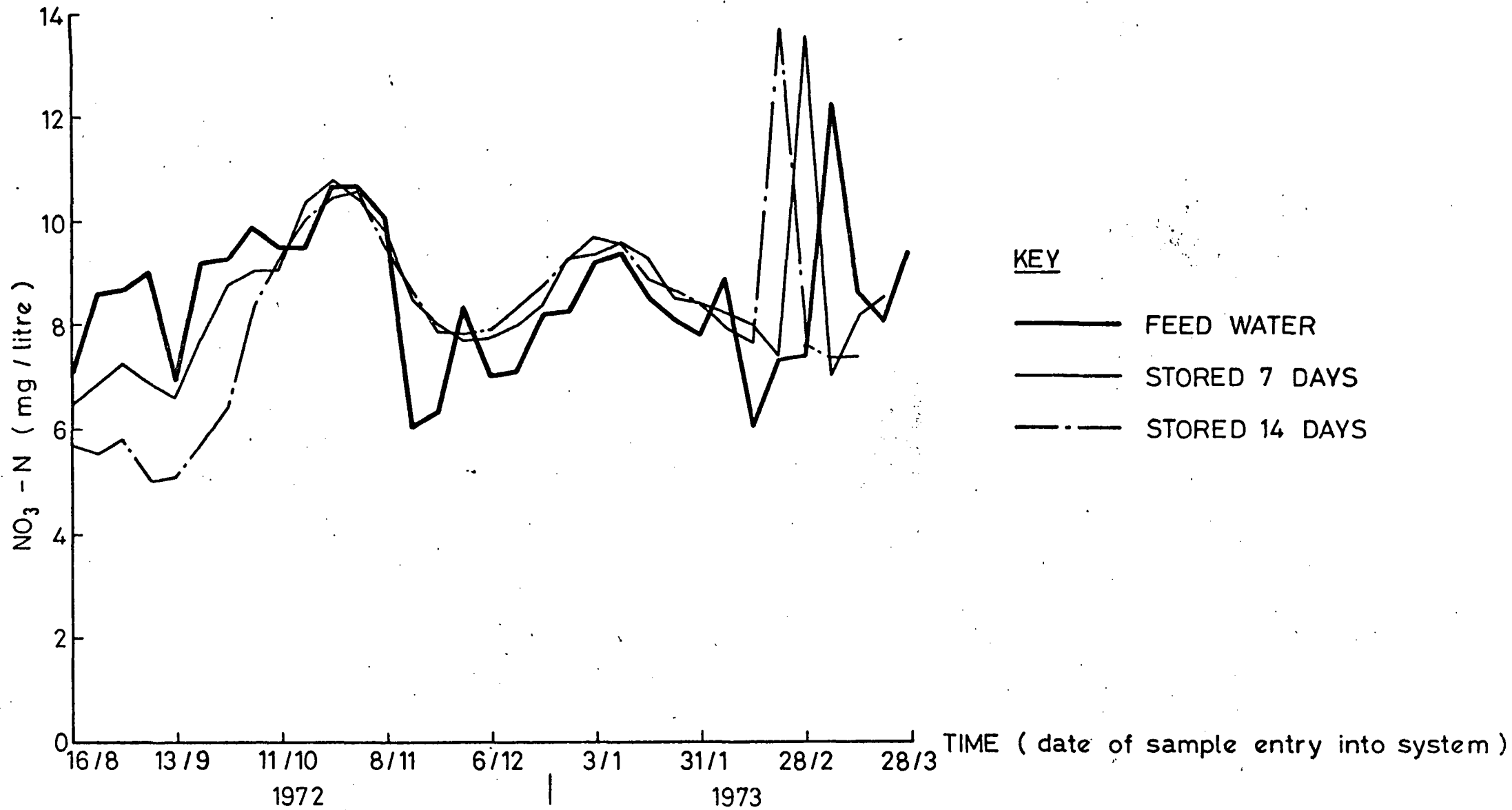FIG.4. MODIFIED STORAGE SYSTEM FOR TRENT WATER AT COLWICK

FIG.5. EFFECT OF STORAGE ON NITRATE - MODIFIED SYSTEM