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Oxidative Nitrogen Fixation in Ultrasonic Field

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The chemical mode of action of ultrasonic waves is not yet explained. The problem generally concerns reactions in aqueous solutions when the chemical changes are regarded as caused by formed hydrogen peroxide, nitrous acid, nitric acid [1-3] and, in addition, possibly ozone [4]. The presence of these substances in irradiated water was explained by Frenkel [5] and Bresler [6], by assuming that during the formation of the cavitation bubble the ionic charges are disrupted and free opposite charges appear on the inside wall of the bubble. These cause great potential differences, resulting in electrical discharge across the gases inside the bubble in diminished pressure.

It can be assumed that activation of gases, and hence chemical reactions between them, takes place in the cavitation bubbles. This activation has not been characterized more closely.* The observation of Loiseleur [7] that the formation of hydrogen peroxide in the ultrasonic field depends on the acidity of the solution is, in this connection, of special interest. According to him, the optimum acidity lies on both sides of the neutral point. pH 4 forms a limit, below which the formation is strongly diminished in proportion to the increase of hydrogen ion concentration.

In the present paper an attempt is made to examine the factors affecting the oxidative nitrogen fixation in the ultrasonic field.

METHODS

Ultrasonic waves were produced by means of a piezo-quartz with a frequency of about 300 kc/sec and with a radiating surface 42 mm in diameter giving a radiating intensity of $\sim$ 10 W/cm^2 in the radiating point. The quartz was completely immersed in an oil bath,

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* *Addition to proof.* Results have also been recorded according to which the reactions in the ultrasonic field can be independent of cavitation. Die erste Ultraschalltagung in Erlangen, May 2–5, 1949 [*Naturwissenschaften* **37** (1950) 14].

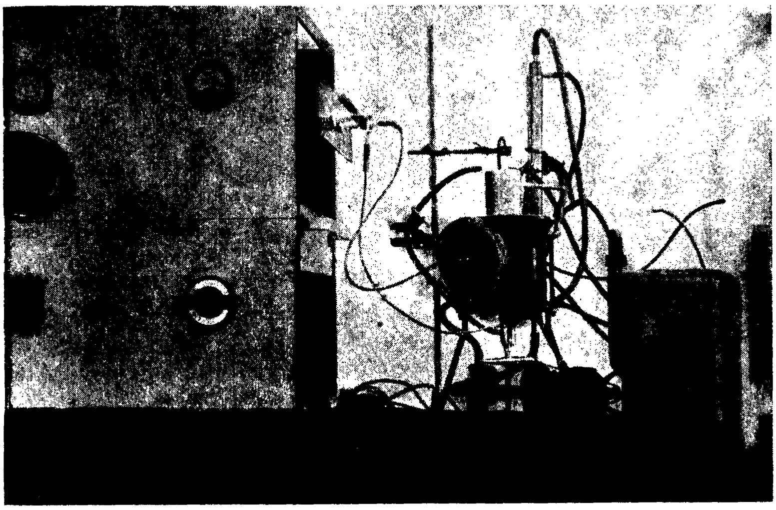

Fig. 1. The ultrasonic apparatus used in the experiments.

from which the vibrations were led into a test cylinder 64 mm in diameter. To avoid loss of energy at the junction, a 0.02 mm steel membrane was used as a bottom for the cylinder ($\lambda/2 \approx 0$). All the experiments with hydrogen were carried out in a glass cylinder to eliminate possible activation of hydrogen on the surface of the metal. The distance from quartz to the bottom membrane was always adjusted to a multiple of $\lambda/2$. All experiments were made at 18—20° C. Fig. 1 illustrates the apparatus used. It was constructed by Mr. J. Nukari in the laboratory of Technical Physics at the Finland's Institute of Technology.

Nitrite nitrogen was determined photometrically using the sensitive colour reaction with α-naphthylamine and sulphanilic acid according to Blom [8]. After 30 min, the reddish colour was estimated with a Klett-Summerson photometer, using the filter S50. The amount of nitrite nitrogen was read from a calibration curve constructed with known concentrations of nitrite.

Hydroxylamine nitrogen was determined colorimetrically according to the technique of Blom modified by Csáky [9].

Nitrate nitrogen was determined photometrically by the phenol disulphonic acid method according to Burström [10]. The yellow colour of the ammonium salt of nitrophenoldisulphonic acid was estimated with a Klett-Summerson photometer using the filter S42. Nitrate nitrogen was read from a calibration curve constructed with known concentrations of nitrate.

Hydrogen peroxide was determined by the titanium sulphate method [11]. The yellow colour was estimated with a Beckmann spectrophotometer (4100 Å). The amount of hydrogen peroxide was read from a calibration curve constructed with known concentrations of hydrogen peroxide.

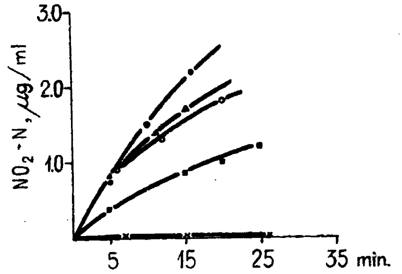

Fig. 2. The amount of NO_2*-N found in aqueous solution after irradiation. Effect of pH on the formation of* NO_2*-N from* N_2*.*

● *pH 11.20*
▲ » *8.00*
○ » *6.20*
■ » *3.30*
× » *1.62*

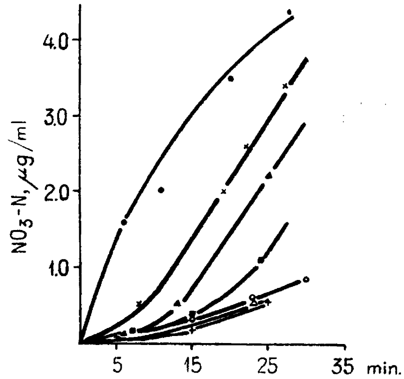

Fig. 3. The amount of NO_3*-N found in aqueous solution after irradiation. Effect of pH on the formation of* NO_3*-N from* N_2*.*

● *pH 1.25*
× » *1.85*
▲ » *2.40*
■ » *3.00*
○ » *3.40*
△ » *12.06*
+ » *7.70*

Ferrous iron was determined by the $a-a'$-dipyridyl method[12]. The reddish colour was estimated with a Beckman spectrophotometer (5000 Å). The calibration curve used in the photometric reading was constructed with known concentrations of ferrous iron.

EXPERIMENTS AND RESULTS

100 ml of distilled water was used in the experiments, and a free access of air was allowed to the test cylinder. Thus, whenever this cylinder was used, the solution contained both nitrogen and oxygen regardless of whether some other gas was also led to the solution. The experiments were chiefly carried out in a steel cylinder. For the sake of control, one experiment was performed in a glass cylinder. The results were similar in both cases. Nitrogen fixation was examined in solutions, the pH values of which ranged from 1.25 to 11.8. The acidity was adjusted to pH between 1.25 and 3 by sulphuric acid, to pH between 3 and 5 by acetate buffer, to pH between 5 and 8 by phosphate buffer, and to pH over 8 by phosphate *plus* sodium hydroxide.

Hydroxylamine could not be found in any experiment.

The curves in Fig. 2 illustrate the formation of NO_2-N at different pH-values.

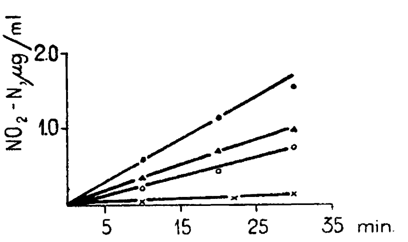

Fig. 4. Inhibitory effect of gaseous hydrogen on the formation of NO_2-N from N_2 during irradiation. pH of the solution in each experiment about 6.8.

● N_2 (45.5 l/30 min)
▲ $N_2 + H_2$ (47.5 l/30 min 6.3% H_2)
○ $N_2 + H_2$ (54.5 l/30 min 11.0 % H_2)
× $N_2 + H_2$ (56 l/30 min 24.8 % H_2)

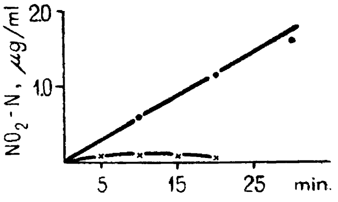

Fig. 5. Inhibitory effect of carbon monoxide on the formation of NO_2-N from N_2 during irradiation. pH of the solution in each experiment about 6.8.

● N_2 (45.5 l/30 min)
× CO (12 l/30 min)

The curves in Fig. 3 show the formation of NO_3-N at different pH-values.

Fig. 4 shows the inhibitory effect of hydrogen on the nitrogen fixation. In different experiments, hydrogen was led into the water (pH~6.8), kept in an open test cylinder in the ultrasonic field, either as pure hydrogen gas or as a mixture of nitrogen and hydrogen. The treatment of different experiments can be seen from the figure.

Curves in Fig. 5 illustrate the inhibitory effect of carbon monoxide on the nitrogen fixation.

Ether prevents nitrogen fixation completely even in small concentrations (0.08 ml/100 ml water). Ammonia weakens the fixation but does not entirely check it even in high concentrations.

The curve in Fig. 6 shows the reduction of ferric iron caused by hydrogen in the ultrasonic field (63.5 µg ferric iron/ml). When hydrogen was led through a ferric-salt solution, outside the ultrasonic field, no reduction was noted. Nor was ferric iron reduced in the ultrasonic field if hydrogen was not led to the solution.

In addition to hydrogen and carbon monoxide, the effect of argon on the nitrogen fixation was also examined. As can be seen from the curves in Fig. 7, the leading of argon to the reaction solution had no definite effect on the formation of nitrite. Smaller variations in the rate of nitrogen fixation between different experiments may be due to disturbances, caused by the bubbles of gas, to the ultrasonic field.

The curves in Fig. 8 show the dependence of the formation of hydrogen peroxide on the hydrogen ion concentration, in experiments where nitrogen

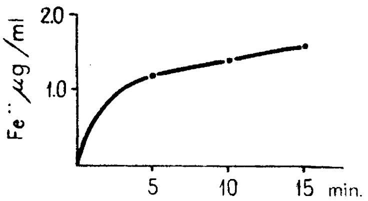

Fig. 6. Reduction of ferric iron to ferrous iron by the effect of ultrasonic waves in the presence of gaseous hydrogen. pH of the solution about 6.8.

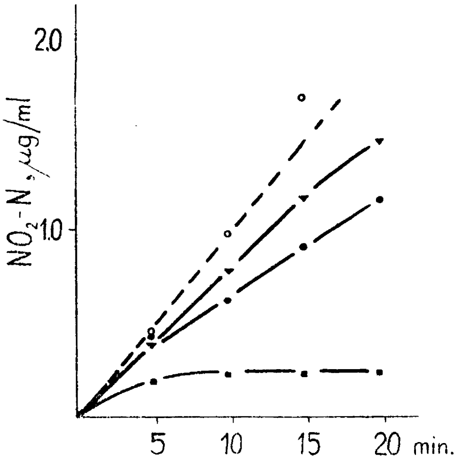

Fig. 7. The effect of gaseous argon and hydrogen on the formation of NO_2-N from N_2 during irradiation. pH of the solution about 6.8.
The full-line curves represent the results of a connected series of experiments, the interrupted curve a separate experiment with argon with maximum nitrogen fixation.

■ *H_2 (30 l/30 min)*
● *A (22 l/30 min)*
▼ *Control, no hydrogen, no argon*
○ *A (20 l/30 min)*

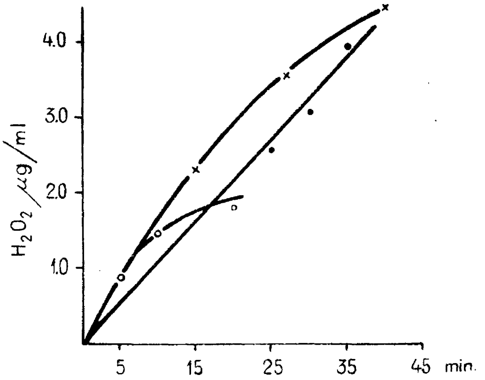

Fig. 8. Formation of hydrogen peroxide at different pH by the effect of ultrasonic waves in the presence of H_2 and CO.

× *H_2 pH 6.85*
● *H_2 » 1.25*
○ *CO » 6.50*

fixation was prevented by hydrogen or by carbon monoxide. Within 30 minutes 30 l of hydrogen or 12 l of carbon monoxide were led into the solution.

Nitrogen fixation is directly dependent on the gaseous oxygen in the solution. If the reaction cylinder is closed, so that oxygen can be removed by boiling and by leading nitrogen gas, washed with alkaline pyrogallol, through the solution, the nitrogen oxides, formed by the effect of ultrasonic waves in the nitrogen atmosphere under normal pressure, amount only to about 8 % of those formed in the same test cylinder in ordinary air atmosphere under normal pressure. Even this minimum nitrogen fixation the authors are inclined to ascribe to residues of oxygen gas. The closed reaction cylinder was similar to that used by Schmid and Rommel [12b].

DISCUSSION

The results obtained in the experiments show that the fixation of molecular nitrogen in the ultrasonic field does not, at least essentially, depend on the hydrogen ion concentration of the solution, as far as the total amount of fixed nitrogen (nitrite *plus* nitrate N) is concerned. Instead, the mutual relation of nitrite and nitrate N is decided by the pH of the solution. In a strongly acid reaction (pH 1—2) the amount of nitrite-N is almost nil, while that of nitrate-N is high. As the acidity is lowered, nitrite nitrogen rises and reaches its maximum in a strongly alkaline reaction. On the other hand, the formation of nitrate nitrogen decreases as the pH rises. At pH 3, the drop is abrupt and at pH 3.4 the quantity of nitrate is already slight and remains so until a strongly alkaline reaction.

These results explain the observation of Loiseleur [7] on the rapid lessening of the formation of hydrogen peroxide below pH 4. The question is thus evidently not one of a decrease in the formation of hydrogen peroxide, but one of the consumption of hydrogen peroxide in the oxidation of nitrite to nitrate. This idea gained evidence by the findings on the inhibitory effect of hydrogen and carbon monoxide on the nitrogen fixation. When the formation of nitrite-N is prevented or strongly weakened, hydrogen peroxide is formed in almost equal quantities at pH 1.85 and 6.85. The hydrogen peroxide formed in the presence of hydrogen is stabile. The analytical values do then not change at the standing of the solution as regularly happens if the nitrogen fixation is not prevented. This instability of hydrogen peroxide was earlier considered to be due to the formation of radicals [6].

The formation of hydrogen peroxide, in the presence of hydrogen, explains also the findings of Mohren [13], according to which the reductive ability of ascorbic acid may rise up to 160 per cent in the presence of hydrogen in the ultrasonic field. The question is evidently one of the reducing effect of the hydrogen peroxide formed. The fixation of nitrogen to nitrite in the ultrasonic field must always be considered when experiments are made in the presence of air.

Our findings on the inhibitory effect of ether on the nitrogen fixation in the ultrasonic field is in agreement with the idea of Frenkel[5] of electric discharges across the gases inside the cavitation bubble in diminished pressure.

The inhibitory action of hydrogen on the nitrogen fixation in the ultrasonic field is of special interest to the authors, because in the aerobic nitrogen fixation by Azotobacter (Wyss and Wilson[14]) and by leguminous root nodules (Wilson and Umbreit[15]), hydrogen also prevents nitrogen fixation. Virtanen[16] has suggested that the first phase in the biological aerobic nitrogen fixation is oxidation: $N_2 \rightarrow N_2^+$ or $N_2 \rightarrow 2N \rightarrow 2N^+$. The transfer of electron to oxygen which possibly is effected by a hemin catalyst system would lead to the formation of nitrogen oxide. The preventive action of hydrogen would then depend on the competition between nitrogen and hydrogen for oxygen. Therefore, it was held important to find out the mechanism of the combination of nitrogen to oxygen in the ultrasonic field and the mode of action of hydrogen on this reaction.

It has frequently been stated already in the beginning of this century that the activation of the nitrogen in the arc process is the most important process[17-19] but it was suggested that parallel activation of oxygen was necessary. The opinion that only oxygen was activated was also presented[20]. Schwab and Loeb[21] considered that nitrogen became reactive after a collision with excited oxygen. Wansbrough-Jones[22] examined the reaction between nitrogen and oxygen in the triode when the purely thermal synthesis of nitrogen oxides was too slow to affect the results. By means of different gas mixtures and by following the diminishing of pressure during the reaction he arrived at the result that the primary reaction in the combination of nitrogen to oxygen is ionization of nitrogen molecule. Activation of oxygen is not necessary. No reaction occurs, unless the electron has an energy greater than 17 volts. Westhaver and Brewer[23] examined the formation of nitrogen dioxide in the glow discharge and also concluded that nitrogen alone is activated. The reaction is initiated entirely by N_2^+ ions. Argon, in the gas mixtures, has a pronounced retarding action on the synthesis of nitrogen dioxide. Westhaver and Brewer explain the influence of argon as follows. The ionization potential of argon lies between that of nitrogen and oxygen, hence, the possibility exists for an A^+ ion to transfer its charge to oxygen by collision of the second kind, i.e., $A^+ + O_2 = A + O_2^+$. This possibility does not exist with nitrogen. Argon, therefore, when added to a nitrogen-oxygen mixture, will markedly decrease the ratio of N_2^+ to O_2^+. The decrease in N_2^+ ion production corresponds to the decrease in the observed rate of synthesis.

Since the ionization energy is most important in the synthesis taking place in the electric discharges, some values for the ionization and dissociation energies of the molecules used in the gas mixtures in the present work are recorded in Table 1.

Table 1. Ionization and dissociation potentials (eV) of hydrogen, oxygen, nitrogen, and argon.

$H_2 \rightarrow H_2^+$	15.4[24] 15.4[25]	$O_2 \rightarrow O_2^+$	12.1[24] 11.7[25]	$N_2 \rightarrow N_2^+$	15.6[24] 15.8[25]	$A \rightarrow A^+$	15.7[23]
$H_2 \rightarrow 2H$	4.5[24]	$O_2 \rightarrow 2O$	5.1[24]	$N_2 \rightarrow 2N$	9.8[24]		
$H \rightarrow H^+$	13.6[24] 13.5[25]	$O \rightarrow O^+$	13.6[24] 13.6[25]	$N_2 \rightarrow N^+$	14.5[24] 14.5[25]		
$H_2^+ \rightarrow H + H^+$	2.6[24] 2.5[25]	$O_2^+ \rightarrow O + O^+$	6.5[24] 7.0[25]	$N_2^+ \rightarrow N + N^+$	8.7[24] 6.9[25]		

If the formation of nitrogen oxides in the ultrasonic field occurs as a result of the ionization of nitrogen in analogy with the ideas presented above, the inhibitory effect of hydrogen could be explained in the same way as Westhaver and Brewer explained the inhibitory effect of argon. The ionization potentials of argon and hydrogen lie near to each other.

The following reactions between nitrogen and oxygen in electric discharges should be possible according to the investigations of Wansbrough-Jones and Westhaver and Brewer:

$$\begin{array}{ll} N_2^+ + O_2 = 2NO & N_2^+ + O_2 = NO_2^+ + N \\ N^+ + O_2 = NO_2 & NO_2^+ + O_2^- = NO_2 + O_2 \\ N_2^+ + O = N_2O & N_2^+ + 2O_2 = (N_2O_4)^+ \\ N^+ + O = NO & (N_2O_4)^+ + e = 2NO_2 \end{array}$$

In our experiments in the ultrasonic field, however, argon does not, at least appreciably, prevent nitrogen fixation. Since, on the other hand, the inhibitory effect of hydrogen is very strong, and under the experimental conditions the hydrogen molecule has been proved to dissociate into atoms, as the reduction of trivalent iron to divalent one shows, the experimental material so far does not give any proof for the idea that the primary reaction in the combination of nitrogen to oxygen in the ultrasonic field is ionization of nitrogen molecule. Harteck [26] found that the atoms of hydrogen and oxygen are very active in many chemical reactions, i. e., they bring about a number of reductions or oxidations. Harteck and Roeder [27] showed that atomic nitrogen reacts with hydrogen peroxide at room temperature forming nitric acid. It is possible that the dissociation of nitrogen and oxygen molecules into atoms in the ultrasonic field is sufficient to cause the combination of nitrogen to oxygen. Thus, the course of the reaction would then be

$$\begin{tikzcd}
N_2 \arrow[r] & N \arrow[dr] & \\
& & NO \\
O_2 \arrow[r] & O \arrow[ur] &
\end{tikzcd}$$

However, it cannot be concluded from our results to what extent the atoms or ions participate in the reaction *, although the combination of nitrogen atom to oxygen atom in the ultrasonic field is very possible.

Our observations on the indispensability of gaseous oxygen for the formation of nitrogen oxide strongly support the idea that nitrogen fixation takes place in the cavitation bubbles. The findings of Grabar [29] on the occurrence of different oxidation reactions in the ultrasonic field even in the complete absence of oxygen from the system do not fit into the formation of nitrogen oxide. The OH radicals arising secondarily from water are evidently unable to oxidize nitrogen.

In the absence of oxygen even hydrogen peroxide could not be detected in our experiments.

SUMMARY

The total amount of oxidatively fixed nitrogen (NO_2-N + NO_3-N) in the ultrasonic field does not essentially depend on the hydrogen ion concentration of the solution. In a strongly acid reaction nitrite-N is oxidized to nitrate-N, and therefore below pH 3 chiefly nitrate-N is formed. Hydroxylamine is not found at any acidity.

Hydrogen inhibits nitrogen fixation in the ultrasonic field, as does carbon monoxide. In the presence of hydrogen, hydrogen peroxide is formed in equal amounts below pH 4 as in neutral reaction, because hydrogen peroxide is then not consumed in the oxidation of nitrite-N.

Argon does not inhibit nitrogen fixation. — Ether inhibits already in low concentrations.

Gaseous oxygen is indispensable for the nitrogen fixation as also for the formation of hydrogen peroxide.

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* Although the dissociation of hydrogen molecule into atoms should be theoretically possible by electrons of 4.5 volts' energy, Glockner, Baxter and Dalton (*J. Am. Chem. Soc.* **49** (1927) 58) found that the reaction begins at 11.4 volts. The dissociation of nitrogen in the ultrasonic field is thus also a natural reaction. Dissociation of oxygen appears from our finding that carbon monoxide prevents nitrogen fixation. According to Geib and Harteck [28] atomic oxygen oxidizes carbon monoxide.

Ferric iron is reduced to ferrous iron in the ultrasonic field in the presence of gaseous hydrogen. On the basis of this, it may be concluded that hydrogen is dissociated to atoms under such conditions. The dissociation of nitrogen is *ex analogia* probable.

The mechanism of nitrogen fixation occurring in the ultrasonic field has been discussed and compared with the corresponding mechanism of aerobic nitrogen fixation by micro-organisms.

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