Modern Biofuel Cells for Waste Recycling In Life Support Systems

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Summary

* A biotechnology approach has been developed for treatment and recycling of waste water involving the construction of a flow-through reactor containing the enzyme catalyst urease.

* A new method for the immobilization of urease has been developed whereby the enzyme is incorporated into a polymer matrix consisting of bovine serum albumin covalently cross linked to the enzyme with glutaraldehyde.

* The polymer has good mechanical properties for use in flow-through reactors and offers long term stability (up to 2 months).

* An immobilized enzyme reactor containing 160 mg of urease is able to treat 1.3×10^{-4} mol urea/min at 25°C.

* The rate of enzyme hydrolysis can be enhanced 4-fold at 65°C.

* Design parameters for enzyme reactors or use in waste water reprocessing have been determined; to eliminate urea from 400 liters of recovered hygiene water per day containing 84000 ppb urea, an enzyme reactor of approximately 500 cm^3 and weighing less than 100 g is required.

* A catalyst bed has been constructed containing Ru coated Al pellets and placed for decomposition of NH_2 into N_2 and H_2.

* A SPE hydrogen-oxygen fuel cell has been placed in combination with the enzyme and catalytic treatment reactors for the generation of electrical from hydrogen evolved during the waste treatment process.

* A new method for treating urea has been discovered based on electrochemical reduction on lead and nickel electrodes at -1.35 and -1.4 V vs NHE.

* High value, low molecular weight substances such as methanol and formaldehyde and ammonia can be gained from this waste treatment process which has good commercial possibilities.

Introduction

Human urine consists of inorganic ions such as Na^+, Cl^-, $H_2PO_4^-$, Ca^{2+}, Mg^{2+}, and organic metabolites of which urea is the main component. Urea is an important contaminant of hygiene water and is present in concentrations in the millimolar range in shower water recovery system for manned space flight [1,2]. Whereas the inorganic ions can be removed from urine and waste water by a variety of purification treatments [3], urea is difficult to remove from waste water, for instance, it cannot readily be separated by chromatography or ion-exchange methods and, unlike most organic contaminants of waste water, it cannot be adsorbed onto activated carbon. Consequently, innovative ways of treating urea are important in the development of water reprocessing technology for long duration space exploration. Urea is a very stable molecule and there are few effective ways for its decomposition [4]. Super critical water oxidation can be used but this requires extreme conditions of temperature and pressures [5]. Ozone in combination with UV light has been reported to be effective in the treatment of urea [6] as are a number of electrochemical techniques [7,8,9] which have attracted attention recently as a means of treating kidney failure.

One approach to urea treatment for water purification is to utilize the enzyme urease to catalyze the hydrolysis of urea to ammonia and carbon dioxide as shown below;

$$NH_2CONH_2 + H_2O \xrightarrow{\text{Urease}} CO_2 + 2NH_3$$

Ammonia (or ammonium ions) produced from this reaction can then be treated by a variety of methods including ion exchange [see e.g.7]. Ammonia can also be utilized as a fuel in electrochemical systems, thus, the treatment of urine with the enzyme urease acts as a convenient step in the purification and recycling of waste water and opens the possibility of generating electrical energy during the waste reprocessing step using a biofuel cell approach [10,11,12].

The approach undertaken in the initial phase of research has been to develop an experimental reactor in which urease can be utilized for urine processing. Enzymes have advantages for use in performing chemical transformations in that they often are highly catalytic, selective for desired reactions rather than producing a large number of unwanted by products and are generally inexpensive to obtain. Consequently, enzymes are used extensively in many commercial and industrial processes. However, it is the ability of the enzyme to perform its catalytic hydrolysis under mild conditions (ie ambient temperatures and pressures), without the need for complex apparatus control mechanisms or a large outside energy source and in a aqueous environment that makes this approach attractive for use in space. A major obstacle to the use of enzymes is that they often exhibit a limited catalytic life time and stability, however, the life time of enzymes can be greatly enhanced through the use of enzyme immobilization techniques (ie where the enzyme molecules are attached to a solid support [for review see 13 & 14]. Numerous catagories of techniques to effect enzyme immobilization have been developed including: (a) ionically bonding to an inert substrate (b) attachment to a insoluble matrix by adsorption (c) physical entrapment in a gel and, (d) covalent attachment to a solid support.

Research Plan

The system being developed during this research consists of 3 treatment phases: (1) urea hydrolysis (2) ammonia catalysis to hydrogen and nitrogen (3) hydrogen/oxygen fuel cell for electricity production. Phase 1 has been completed and a water treatment column has been constructed for urine treatment; the research strategy has been to assess the feasibility of immobilized urease for urine treatment and waste water treatment. Phase 2 has involved taking the ammonia produced, separating it from aqueous solution to form ammonia gas and passing it over a Ru catalyst bed to from nitrogen and hydrogen as shown below:

$$2NH_3 \rightarrow N_2 + 3H_2$$

The performance of the catalyst bed is being investigated at the present time. Phase 3 will involve taking the mixture of hydrogen and nitrogen and passing it to a fuel cell. A solid polymer fuel cell commercially available from Electrosynthesis Company (New York) has been included as the final stage in the waste processing stream.

$$3H_2 + 1.5O_2 \rightarrow 3H_2O$$

A schematic diagram of the apparatus set up in the laboratory is shown in Figure 1.

Methodology

Enzyme Activity: Definitions

Urease activity:

1 unit can generate 1 mmol NH_3 in one minute where an excess of $CO(NH_2)_2$ is present (usually >0.1M).

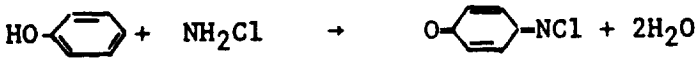
Specific activity of urease:

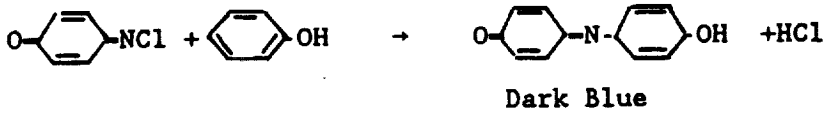
number of active units in 1 g of enzyme protein.

The enzyme activity towards urea was assessed by determination of the NH_3 that is evolved by a standard amount of enzyme in a certain period of time.

Ammonia production was monitored by UV-Vis spectroscopy according to published procedures [15]. Two color developing reagents are used in this assay S_1 (0.5M phenol + 0.001 sodium nitroprusside) and S_2 (0.65 M NaOH + 0.03 M NaOCl). S_1 and S_2 form a dark blue product with NH_3 and is proportional to the absorbance at λ = 625 nm. A calibration curve showing absorbance (625 nm) against concentration of NH_3 is shown in Figure 2. This reaction sequence is sumarised below:

1. $NH_3 + OCl^- \rightarrow NH_2Cl + OH^-$

2. 

3. 

Dark Blue

Results

Results Enzyme Activity

Sigma Type III urease was used in these experiments EC 3.5.15. (13,000 μ unit/g). Urease (70 mg) was dissolved in 100 ml of 0.02M phosphate buffer containing 1g of EDTA pH 6.36. EDTA was included to protect the enzyme from trace amounts of heavy ions eg Cu^{2+} and Pb^{2+} which can have a harmful effect on the enzyme. Urea solution contained 15 g $CO(NH_2)_2$ in 1000 ml (0.25M). The enzyme activity was assayed at 23 ^{o}C and the results are given in Table 2. A graph showing absorbance versus time is given in Figure 3.

Temperature Dependence of Urease

By making these measurements, it is possible to determine the optimal temperature for the enzyme-catalyzed hydrolysis of urea and information concerning the kinetics of the enzyme reaction. Results are given in Table 3. Manufacture's specification are 13,000 units/g of protein, however, these results indicate 1,800 units/g. A possible explanation is that some enzyme activity has been lost either in storage or during the course of the experimental procedures. Figure 4 shows is a plot of enzyme activity versus temperature. The activity of the enzyme increases up until 65^{o}C; above this temperature, the enzyme activity decreases because of heat denaturization.

Figure 5 is a plot of log V_{max} versus T^{-1}. The slope of the plot is as follows:

Slope b. $$\frac{d\ \ln V}{d\ 1/T} = -1.65 \times 10^{3}$$

Using the relationship:

$$K = A\, e^{\frac{-Ea}{RT}}$$

because the reaction is zero order V = K hence:

$$\ln V = \ln A - \frac{Ea}{RT}$$

$$\frac{d \ln V}{d\, 1/T} = \frac{-Ea}{R}$$

Thus:

$$\frac{-Ea}{R} = -1.65 \times 10^3$$

$$Ea = 13.7 \text{ kJ/mol} \quad (3.27 \text{ kcal/mol})$$

The activation energy lies in the range expected for a diffusion-controlled process. The reaction sequence catalyzed by urease may be summarized as follows:

$CO(NH_2)_2 \rightarrow$ Urease (substrate diffuse to enzyme)

Urease + $CO(NH_2)_2 \rightarrow$ Urease-$CO(NH_2)_2$ (enzyme-substrate complex)

Urease-$CO(NH_2)_2$ + $2H_2O \rightarrow$ Urease + HCO_3^- + NH_3 + NH^{4+}

HCO_3^- + NH_3 + $NH^{4+} \rightarrow$ Urease (substrate diffuse from enzyme)

The results indicate that diffusion of urea to the enzyme or reaction products from the enzyme is the rate determining step for the reaction.

Immobilization Procedure

A review of the literature concerning the immobilization of urease has been undertaken. A vast array of methods have been established for urease immobilization, however, most of the published literature is focused on the use of urease in clinical analysis where relatively small amounts of the enzyme are used [see e.g. 10] thus making many of these methods unsuitable for the bulk processing approach needed for water recycling. Consequently, other methods were assessed for their suitability for this project; the methods were assessed from the following view points (a) achieving long-term stability (ie activity) of the enzyme (b) providing material with strong mechanical properties (c) the immobilized enzyme must low solubility for use in flow through reactor.

A proteic-polymer methods described by Cocquempot et al [16,17] was assessed to be a highly suitable approach for this work. This method involves mixing the enzyme with another protein (bovine serum albumin) then adding a chemical cross linking reagent (glutaraldehyde) which chemically binds the urease and the BSA covalently through the -CHO groups of glutaraldehyde with the $-NH_2$ groups of the proteins such that a polymer is formed. The polymer has a sponge-like texture and is somewhat porous. The method was originally found to be effective in the enhancement of the stability of plant photosynthetic enzyme complexes and thus seemed to have good prospects for enhancing the catalytic lifetime of the enzyme urease. A coplymerization method was chosen because of its suitability for enzyme immobilization in a column reactor since the enzyme will not be washed away in a flowing urea solution and it provides a high contact surface with flowing urea solution: a schematic representation of the cross-linking process is given in Figure 6.

Urease was dissolved in a 100ml buffer solution containing 0.02 KH_2PO_4/K_2HPO_4 and 0.2g EDTA at pH 7.0. EDTA forms a complex with contaminating heavy metal ions thus protecting the enzyme from possible harmful effects.

The procedure for forming batches of immobilized urease involved mixing the following materials sequentially in a 15 ml test tube.

5.0 ml (400mg/100ml) urease solution,

2.5 ml 24% bovine serum albumin

2.5 ml 1.5% glutaraldehyde

The mixing was carried out at -25°C by placing the test tubes in a dry ice saturated with NaCl. The mixtures were left at this temperature for 4 hours. The tubes were then transferred to at -5°C freezer for a further 2 hours. The material at this stage forms a brown colored polymer which is sponge-like in appearance. The material can be stored in buffer solutions at temperatures of -5°C for extended periods of time; preliminary measurements show that the enzyme remains catalytically active after 2 months of storage. Large amounts of this material is in storage for future use.

Enzyme Column Fabrication

The approach described above has been used to make large amounts of polymer containing immobilized enzyme. The next stage of the research has been to construct a water-treatment column containing the immobilized urease.

A glass column was packed with urease-containing proteic polymer as shown schematically in Figure 7. Before packing the column, the mixture was taken from the freezer and placed in a refrigerator where it was left to stand at for 3 hours at 4 °C. This material was then broken down into small pieces and

used as column packing materials. Glass wool was placed at the bottom of the reactor to prevent the escape of the enzyme material. when the column was packed with the enzyme, the volume of solution it contained was 200 ml.

Activity of Immobilized Enzyme

The procedure to determine the catalytic activity of the immobilized enzyme first involved washing the column thoroughly with double distilled water. A liter of urea solution was made up containing 15 g urea, 0.5g KH_2PO_4, K_2HPO_4, and 0.2 g EDTA at pH 7.0 and was passed through the column at a flow rate of 2ml/min. The reacted solution were collected in 1 ml samples from the bottom the column and assayed for the presence of NH_3. The assayed was performed essentially as described above by adding 4.5 ml of S_1 and 4.5 ml of S_2. A blank was obtained by collecting solution from the initial column washing.

The rate of NH_3 formation under these conditions was 2.6×10^{-4}/min.

The conversion efficiency for $CO(NH_2)_2$ is:

$$\underset{\text{conversion}}{CO(NH_2)_2} = \frac{CO(NH_2)_2 \text{ decomposed per min}}{CO(NH_2)_2 \text{ input per min}} = \frac{1/2 \times 2.6 \times 10^{-4} \times 100\%}{0.25 \times 0.002}$$

$$= 26\%$$

Decomposition of NH_3

The approach adopted in this phase of the research was to determine the feasibility of obtaining hydrogen from ammonia. The reaction has the following thermodynamic characteristics:

$$2NH_3 \rightarrow N_2 + 3H_2$$

$$Ke = \frac{[N_2]\,[H_2]^3}{[NH_3]^2}$$

ΔG^o = 32.88 kJ/mol

ΔS^o = 197.87 J/mol

ΔH^o = 91.88 kJ/mol

These data show that although ΔG^o is positive, ΔS^o is also positive so the equilibrium constant can be increased by increasing the temperature; the effect of increasing temperature on the dissociation of ammonia is given in Table 4. From the table, at reaction temperatures of 350°C-400°C NH_3 can be decomposed to H_2 and N_2. However, NH_3 is a stable molecule and its break down requires a high activation energy and requires a catalyst.

Previous work [18,19,20] has shown that Ru is a particularly good catalyst for the decomposition of ammonia also Fe catalysts are used because of their low cost (see Figure 8). A catalytic ammonia decomposer containing a Ru catalyst has been constructed in this laboratory and is shown in Figure 9 and is based on the design described elsewhere [18]. The column is packed with 1/8 inch alumina pellets coated with Ru (Ru content = 0.5%). Alumina surface area is approximately 2400cm^2/10mg.

This reactor has been designed to have the following characteristics:

Operation temperature	350°C
NH_3 input rate	260 ml/min (1 atm)
NH_3 output	172 ml/min
H_2 output	132 ml/min
N_2 output	194-259 ml/min

Under these conditions, the reactor can provide 9.8 x 10^{-5} mol H_2/s. A fuel cell working at 1A needs 5.2 x 10^{-6} mols H_2/s.

Feasibility Study:

Enzyme Technology Application for Waste Water Treatment

Preliminary calculations on the design of an enzyme bed for the treatment of waste water contaminated with urea. The size and performance of the reactor bed will be based on the following assumptions.

1. Urea concentration in typical waste water = 84000 ppb (ie 1.4×10^{-3}M).
2. A total of 400 liters of water will be processed on a daily basis by the water recovery system.

The amount of enzyme required to maintain this amount of water free of urea can be determined as follows:

Enzyme activity is proportional to the concentration of urea and can be determined from the Michaelis Menton equation based on $K_m = 2 \times 10^{-2}$ M urea.

$$V = \frac{V_{max}[S]}{[S] + K_m} = \frac{V_{max}\, 1.4 \times 10^{-3}}{1.4 \times 10^{-3} + 2.0 \times 10^{-2}} = \frac{1.4}{21.4} V_{max}$$

$$= 0.065\ V_{max}$$

At this low concentration of urea, the enzyme activity is 6.5% of the rate observed with 0.1M urea. Therefore, it is reasonable to expect that the rate of the immobilized enzyme column is reduced by 6.5% in the presence of these lower concentrations of enzyme.

It is possible to predict the rate of urea hydrolysis by the immobilized enzyme column under the lower levels of urea in solution.

$$\text{average enzyme velocity} = \frac{V_o}{2} = \frac{6.5\%\ V_{max}}{2}$$

Under steady state conditions (flow rate is 2 mls/min) the measured enzyme velocity of the immobilized enzyme column was 2.6×10^{-4} mol NH_3/min.

The amount of enzyme used to form the column was 160 mg urease and the efficiency of conversion of urea to NH_3 and CO_2 was 26%.

The amount of urea that this reactor can hydrolyse per day can be determined as follows is

$$= 6.5\% \times 0.5.\ 1.3 \times 10^{-4} \times 60 \times 24$$

$$= 6.084 \times 10^{-3}\ \text{mol/day}$$

However the amount of urea in 400 liters of contaminated water is given below

$$400 \times 1.4 \times 10^{-3}\ \text{mol} = 0.56\ \text{moles}$$

This result indicates that, under these conditions, an enzyme column that is 92 times larger than the one in the lab is necessary for the complete removal urea from 400 liters of contaminated water on a daily basis ie the reactor should include approximately 14.7 g of urease instead of 0.160 g. The volume of the present immobilized enzyme reactor is 196 cm^3 therefore using this technique, a reactor of volume approximately 19600 cm^3 would be required which is unacceptably large, however, the following points have to be taken into account.

1. Type III Sigma urease has a low specific activity and other types of urease having a ten times higher specific activity can be obtained commercially which would reduce the amount of enzyme and the size of the reactor by an equivalent amount.
2. The enzyme is loosely packed into the reactor and significant volume savings can be achieved through a tighter packing of the enzyme in the column.
3. Much of the volume in the column is taken up by the BSA supporting matrix and glutaraldehyde which may not be necessary.
4. Urease is 4 times more active at 65°C than at 25°C at which the

experiments were performed therefore a further reduction can be achieved at slightly raised temperatures (NB heat pasteurization is usually performed at 62.8°C).

Conclusions

It seems reasonable to conclude that a reduction in volume of close to 2 orders of magnitude can be achieved through simple modifications to the present reactor system. A flow-through reactor of less than 500 ml^3 can be highly effective in removing urea for large volumes of recovered hygiene water (ie up to 400 liters). The enzyme can be made more effective if it is combined with low grade heat or is used in combination with water that is at higher temperatures during pasteurization. The weight of the protein contents of a 500 cm^3 enzyme column is approximately 30 grams.

UREA CATHODIC REDUCTION

1. Purpose

Former studies have involved the disposal of urea by anodic oxidation into N_2 and CO_2:

$$2\ H_2O + 2\ CO(NH_2)_2 \rightarrow 2\ CO_2 + N_2 + 12\ H^+ + 12\ e^-$$

in most former studies (21-24). We, however, are trying to find a new method to dispose urea in waste water by reducing it into useful products such as ammonia, methanol and formaldehyde.

2. The Feasibility of This Reaction

Even though there is no study of cathodic reduction of urea in literature, the reduction of a carbonyl group such as ketone or aldehyde is well defined in organic electrochemical synthesis. The current efficiency can be as high as 80-95% (25-31). In a similar fashion we can propose a possible mechanism for cathodic reduction of urea according to that of ketone.

B. Thermodynamic Consideration of The Reaction

The free energy reaction charge and the reversible potential for the reduction of urea will be determined. Two possible reaction pathways will be considered as follows:

Reaction (1)

$$CO(NH_2)_2 + 6H^+ + 6e^- \rightarrow H_2O + NH_3 + CH_3\,NH_2 \qquad (1)$$

$$\Delta G^o\ (298.15\ k,\ 1\ atm) = 6.6 - 6.35 - 56.687 - 0+48.72$$

$$= -7.717\ (kcal/mol)$$

$$E^o(1) = \frac{\Delta G^o(1)}{nF} = \frac{-7.717 \times 10^3 \times 4.184}{-6 \times 96500} = 0.0558V\ (NHE)$$

Reaction (2)

$$CO(NH_2)_2 + 6H^+ + 6e^- \rightarrow 2\ NH_3 + CH_3OH$$

$$\Delta G^o = 2 \times (-6.35) - 39.73 - 48.72$$

$$= -3.71\ kcal/mol$$

$$E^o(2) = \frac{\Delta G^o(2)}{nF} = \frac{-3.71 \times 10^3 \times 4.184}{-6 \times 96500} = 0.0268V\ (NHE)$$

It seems that:

(1) From the calculations it can be concluded that the reaction is thermodynamically favored, however, we can expect that kinetics may play an important role.

(2) The ΔG° values for the two reactions are similar, so the product may be a mixture.

4. Electrolysis

First, the limiting current i_L at large η will be determined. The r.d.s. in this case is diffusion limited. Thus,

$$i_L = - DnF \frac{C_{bulk}}{\Delta}$$

assume $D = 5 \times 10^{-6}\ cm^2\ s^{-1}$

$n = +6$

$F = 96500\ C$

$C = 4M$ (Urea 0-8M)

$\Delta = 10^{-2} - 10^{-3}\ cm$

So, $i_L = -5 \times 10^6\ cm^2\ S^{-1} \times 6 \times 96500 \times 4/1000\ cm^3 \times 5 \times 10^{-3}\ cm$

$= -2.31\ 6A/cm^2$

Second, consider the log i-E diagram

Cathodic reaction: (reduction of urea)

$$i = i_o \exp\left(\frac{-\alpha nF\ (E-Eeq)}{RT}\right)$$

$2.303 \log i = 2.303 \log io - \alpha nF/RT\ \eta \qquad \alpha = 0.5$

$\eta = -1.97 \times 10^{-2} \log i/io.$

Use: $CO(NH)_2 + 6H^+ + 6e^- \rightarrow 2NH_3 + CH_3OH \qquad E^o = 0.268V$

Assume: io for urea is 10^{-8} A/cm^2.

Then:

i(A/cm^2)	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1
η(v)	-0.04	-0.06	-0.08	-0.10	-0.12	-0.14	-0.16

For $2H_2O + 4H^+ + O_2 + 4e^-$ $\quad E^o = 1.22$ V

Anodic Reaction, Oxidation of H_2O

$$2.303 \lg \frac{i}{io} = \frac{0.5 \times 4 \times 96500}{8.31 \times 298} \eta$$

$$\eta = 0.0295 \log i/io$$

Assume io is 10^{-10} A/cm^2

i(A/cm^2)	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1
η(v)	0.12	0.15	0.18	0.21	0.24	0.27	0.30

Then, we plot as Fig. 6.

5. Experimental Work

A. Instrumentation

A modified PINE Instrument RDE4 potentiostat was used for voltage sweep experiments and potentiostatic electrolysis. Current versus potential curves and current versus time curves were recorded on a Hewlett Packard XY recorder (Model 7044B).

Most of the experiments on urea reduction have been performed in a three-compartment cell. The solution was continuously stirred by a magnetic follower.

B. Electrodes

Counter electrode: platinum gauze was used as a counter electrode. The reference electrode was a silver-silver chloride electrode.

Working electrode: we chose different electrode material as the working electrode. Hg, Pb has the highest overpotential for H_2 evolution and Ni, Cu, Fe have medium high overpotential. The reduction process was carried out only on Pb and Ni cathodes.

C. Electrolyte Solutions:

Solutions were made using millipore or triply distilled water and analytical grade chemical.

Selection of Supporting Electrolyte

Several supporting electrolytes such as H_2SO_4, H_3PO_4, HCl, HNO_3 and Na OH were considered H_2SO_4 and H_3PO_4 were rejected because Ce precipitation would occur. Ce $(NO_3)_6^{2-}$ will be used to detect methanol, which is one of the reaction products. In the case of HCl and HNO_3, there is the possibility of chlorine evolution and NH_3 formation respectively. Thus it appears, that NaOH is the most suitable supporting electrolyte.

D. Current/Voltage Studies

In these experiments the change in current (i) as a function of potential (E) were determined. specifically, the applied potential on the working electrode will be increased (in a negative direction) at a constant rate and

the corresponding values of current are recorded. A plot of i versus E will show current peaks coresponding to the potential at which the maximum rate of reduction of the organic substance is occurring. This method represents a convenient means to screen material (e.g., to determine the appropriate reaction conditions). The electrolysis can then be performed at the potentials determined for the reaction.

Constant Current/Constant Potential Electrolysis

These experiments are performed over longer time perios (9 hours) than the voltammetry described above. Constant potential electrolysis is where the working electrode (i.e., the cathode in these experiments) is maintained at a constant potential, and the magnitude of the current, as a function of time, is measured. Using this method, the number of Faradays required to complete the electrolysis of 1 mole of substrate can be determined. Also, selective reduction of different chemical species can be performed. during constant current electrolysis, the potential of the working electrode will vary with time as the concentration of substrate decreases, thus selective reduction would be difficult. Therefore, batch electrolysis at constant current can give quite different products than constant potential electrolysis.

F. Product Analysis

The gaseous products resulting from electrolysis will be analyzed using gas chromatography. From a quantitative assessment of the reaction products, coupled with information of the current that has flowed during the electrolysis, the current efficiency for urea reduction can be assessed. Information concerning the reaction mechanism can be obtained in this way. Analysis of the constituents in the liquid phase will be performed.

G. Detection of Reaction Products (32)

Most of the compounds were monitored by UV-VIS spectroscopy according to published procedure (15, 82).

A. Detection of Methanol

Using cemic ammonium nitrate as reagent, methanol can be detected.

$$(NH_4)_2\ Ce\ (NO_3)_6 + CH_3OH \rightarrow (NH_4)_2\ Ce\ [OCH_3]_6 + CHNO_3$$

red

λ = 486 nm

B. Detection of Formaldehyde (COH_2)

Formaldehyde reacts with chromotropic acid to form a compound detected at λ = 570 nm.

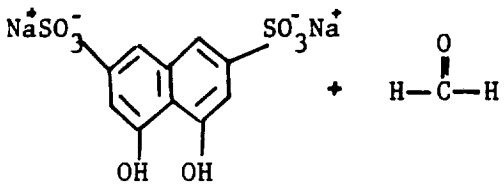
Principle: Using chromotropic acid:

Reagent 1) 10% chromotropic acid solution

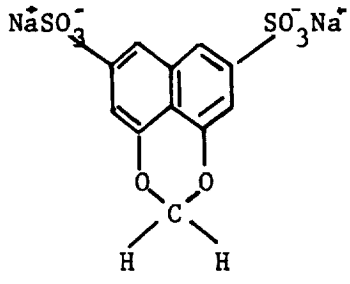
2) 1:1 H_2SO_4: H_2O

3) Concentrated H_2SO_4

Procedure: Take 1 ml sample (~ 0.037 mg/2, 0.037 mg/2, 0.074 mg). Add 1 ml 10% chromotropic acid, add 5 ml concentrated H_2SO_4. Stir violently, heat at 150°C for 0.5 hr, dilute to 50 ml by 1:1 H_2SO_4, measure absorbance with regent blank.



Chromotropic acid



blue

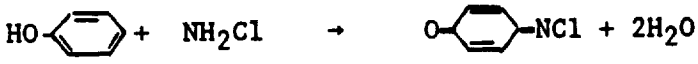
= 570 nm

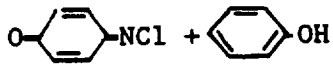
C. Detection of Ammonia: Indophenol Reaction (34)

Two color developing reagents are used in this assay S_1 (0.5 M phenol + o.001 M soldium nitroprunide) and s_2 (0.65 M NaOH + 0.03M NaOCl). S_1 and S_2 form a dark blue product with NH_3 and is proportional to the adsorbance at 625nm. A calibration curve showing absorbance (625 nm) against concentration of NH_3 is then establish this reaction sequence is summarized below.

Indephenol Reaction

1. $NH_3 + OCl^- \rightarrow NH_2Cl + OH^-$

2.  $+ NH_2Cl \rightarrow$ $+ 2H_2O$

3.  $+$ $\rightarrow$  $+HCl$

Dark Blue

H. Experimental Results

Constant Potential Electrolysis of Urea

(1) The concentration of urea in a phosphate buffer solution KH_2PO_4 0.5 M-K_2HPO_4 0.5 M, was 2 M. The pH of the solution was 6.8.

(2) A lead sheet 52 cm^2 (geometric area) was used as the cathode.

(3) The background current at E = -1.0 V was i_c = 1.5 mA

at E = -1.5 V it was i_c = 12 mA

at E = -1.6 V it was i_c = 14 mA

(4) The potential of the lead electrode was set at Ec = -1.35 υ, the limiting current was then i_c = 4.5 mA. After nine hours of electrolysis the limiting current rose to 8.2 mA.

At this potential almost no H_2 is evolved from Pb, but there is some O_2 evolution at the Pt counter electrode.

After several runs of 9 hours of electrolysis the mean concentration of ammonia produced per run in the cathodic compartment was 32.4 x 10^{-3} mole/liter. since the cathodic volume was 68 ml, the total amount of ammonia produced was 0.002206 mole.

Current Efficiency

Assuming 100% current efficiency and having estimated the limiting current, and the ideal amount of NH_3, the current efficiency for the process can be assessed.

According to the reaction:

$$CO(NH_2)_2 + 6H^+ + 6e^- \quad 2NH_3 + CH_3OH \qquad (2)$$

To produce 1 mole of NH_3, three Faradays are required. The production of 0.002206 moles of NH_3 therefore requires 638.12 Coulombs. It is assumed that the limiting current (i_L) throughout the reduction process is the mean value of the limiting current measured at the beginning and the end of the process.

$$i_L = \frac{i_{initial} + i_{final}}{2} = \frac{4.5 + 8.2}{2} = \frac{12.7}{2} = 6.35 \text{ mA}$$

The total amount of electricity consumed during the 9 hour long process is

$$Q = 9 \times 3600 \times 6.35\ 1\ 10^{-3} = 205 \text{ Coulombs}$$

The current efficiency according to the path of reaction (2) would be:

$$\eta = \frac{638}{34} = 300\%$$

This anomalous current efficiency value means that our assumed reaction pathway is less likely to occur.

Let's now consider the first suggested mechanism of reaction i.e.

$$CO(NH_2)_2 + 2\ e^- + 2H^+ \rightarrow \overset{O}{\overset{\|}{C}} + 2NH_3 + H_2.$$

The amount of current required to produce 0.002206 moles of NH_3.

$$Q = \frac{638}{3} = 212 \text{ Coulombs}$$

The current efficiency $\eta = 100\%$, which is more reasonable.

Pb is a good electrode material which has a high overpotential for hydrogen evolution, but cannot catalyze the electrochemical synthesis of methanol or formaldehyde.

CONCLUSIONS

(1) On Pb cathodic electrolysis, we suggest a mechanism which would lead to CO.

(2) We find out qualitatively that there is quite a lot of COH_2 and/or CH_3OH formed if we use Ni gauze as cathode at E = -1.40 V, ic = 95 mA for several hours. This is in agreement with the phenomena of Ni catalyzed hydrogenation. So, it would appear that the reaction mechanism on Ni electrode is:

$$CO(NH_2)_2 + 6H^+ + 6e^- \rightarrow CH_3OH + 2NH_3$$

or $$CO(NH_2)_2 + 4H^+ + 4e^- \rightarrow CH_2O + 2NH_3$$

To know the detailed mechanism, the current efficiency should be determined, followed by the use of GC to detect the product quantitatively, and, thus, propose a preliminary mechanism. Later we would need to use cyclic voltammogram the RRD (Rotating Ring Disk Electrode) or RDE (Rotating Disk Electrode) electrode to establish the complete mechanism.

Economical Feasibility Study

Price of ammonia: - \$40/ton (average)

of methanol: - \$0.98/gallon

Assume: $CO(NH_2)_2 + 6e^- + 6H^+ \rightarrow 2NH_3 + CH_3OH$ on nickel electrode

1 ton NH_3 . 10^6g = 5.88×10^4 mol NH_3

Simultaneously produce => methanol 2.94×10^4 mol CH_3OH => 9.4×10^4 g i.e. => 344 gallon

That is \$326.8 methanol.

Then, suppose our cell potential is 2 V and the price for electricity is \$0.03

per kWh.

Cell potential 2 V.

Energy input = 3.4×10^{10} CV = 3.4×10^{10} J

1 kwh = $10^3 \times 3600 = 3.6 \times 10^6$ J

$$\frac{3.4 \times 10^{10}}{3.6 \times 10^6} \times 0.03 = 0.94 \times 7000 = \$283$$

We can see: produce and sell 1 ton ammonia + 344 gallon CH_3OH we get 40 + 326.8 = \$366.8 the investigation is: \$283.

We can earn \$83.8 per ton of ammonia synthesis by electrochemical reduction of urea.

Suggested Follow-Up Work

* Test activity of immobilized enzyme in presence of low concentrations of urea.
* Test effect of organic and inorganic contaminants on immobilized enzyme activity.
* Assess the long term stability of immobilized enzyme under conditions of continuous use.
* Establish methods to immobilize the enzyme whereby it retains higher rates of urea hydrolysis.
* Examine reactors of immobilized of ureaolytic microorganisms as a means of treating low amounts of urea and of achieving enhanced enzyme stability.
* Test the enzyme reactor with urine.
* Establish methods to enhance electrical energy generation based on enzymatic urea treatment.
* Examine the direct use of electrodes for urea reduction.
* Examine the reaction products for urea reduction using GC analysis and determine the current efficiencies.

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Table 1

Calibration of $[NH_3]$ versus absorbance at λ = 625nm

	1.0	1.5	2.0	2.5
ABSORBANCE	1.109	1.717	2.26	2.783

Table 2

Enzyme Activity Measurements at 23°C

Sample	Blank	1	2	3
Urease (ml)	0.0	0.2	0.2	0.2
Urea (ml)	1.0	1.0	1.0	1.0
Time (min)		5.27	10.53	15.9
Absorbance		0.12	0.749	1.187

Table 3

Activity of Urease Versus Temperature

Temperature °C	13	24	38	50	55	65	75	85	90
absorbance min	0.08	0.10	0.76	0.41	0.43	0.47	0.43	0.33	0.10
ppm NH_3 min	0.07	0.09	0.23	0.37	0.39	0.42	0.39	0.3	0.09
μmol NH_3 mg urease/min	0.3	0.38	0.99	1.56	1.66	1.79	1.64	1.26	0.38
relative activity versus 65°C	16.4%	21.4%	55.4%	87.3%	92.5%	100%	91.6%	70.0%	21.4%
$1/T$ $(10^{-3}K^{-1})$	3.5	3.37	3.22	3.10	3.05	2.96	-	-	-

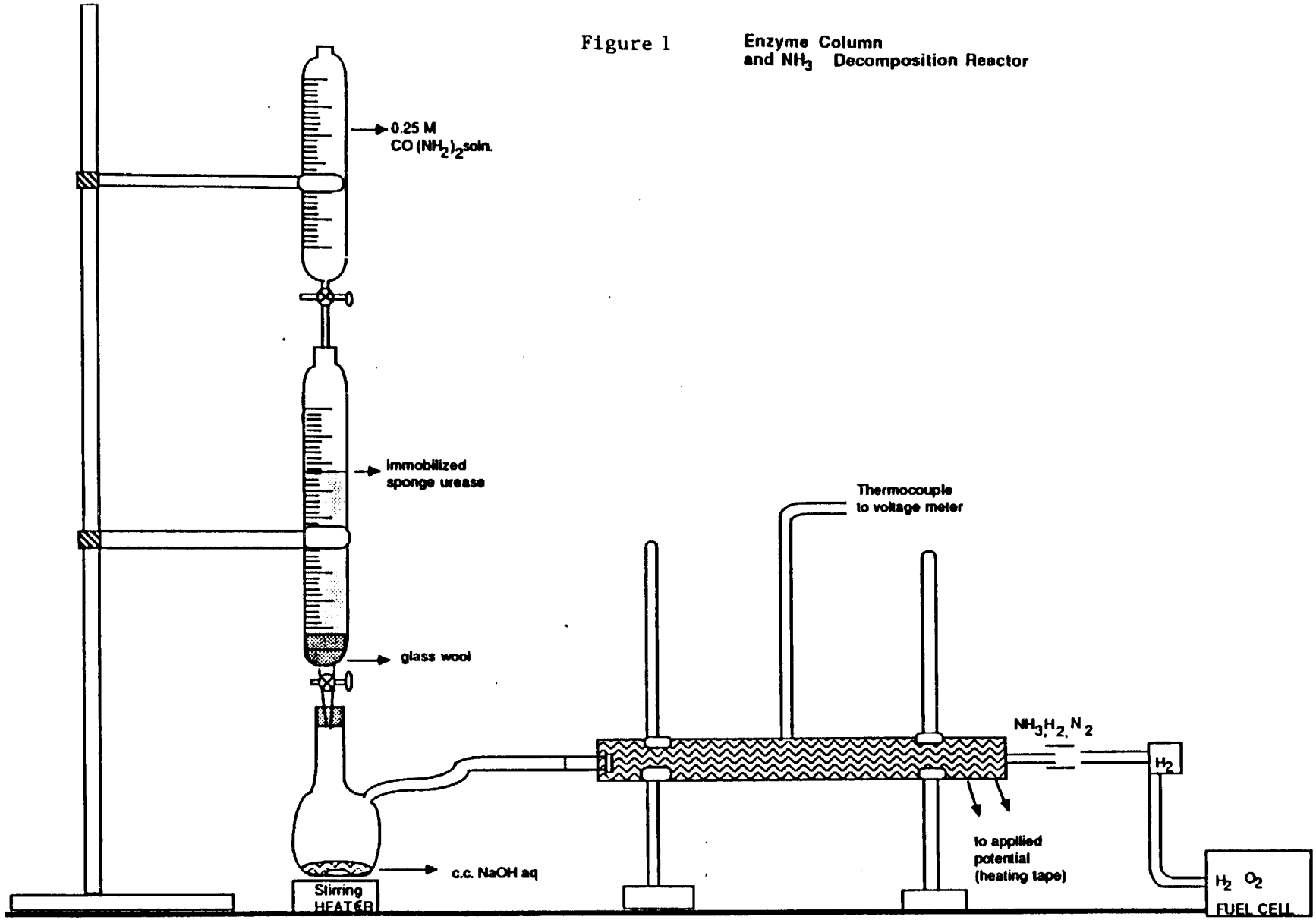
1. Absorbance/min was converted to ppm NH_3 per min using the NH_3 versus absorbance calibration curve shown in Figure 3 (ie 1.103 abs/ ppm (NH_3).

2. 0.2 ml of urease solution contained $\frac{0.2 \times 70mg}{100\ ml} = 0.14$ mg

Table 4

Temperature	ΔG^o (kJ)	log K_{eq}	$\frac{[NH_3] \text{ dissociated}}{\text{total } NH_3}$
25°C (298K)	32.88	-5.76	low
100°C (373K)	18.04	-2.52	67%
200°C (473K)	-1.71	1.84	97%

Figure 1 Enzyme Column and NH_3 Decomposition Reactor



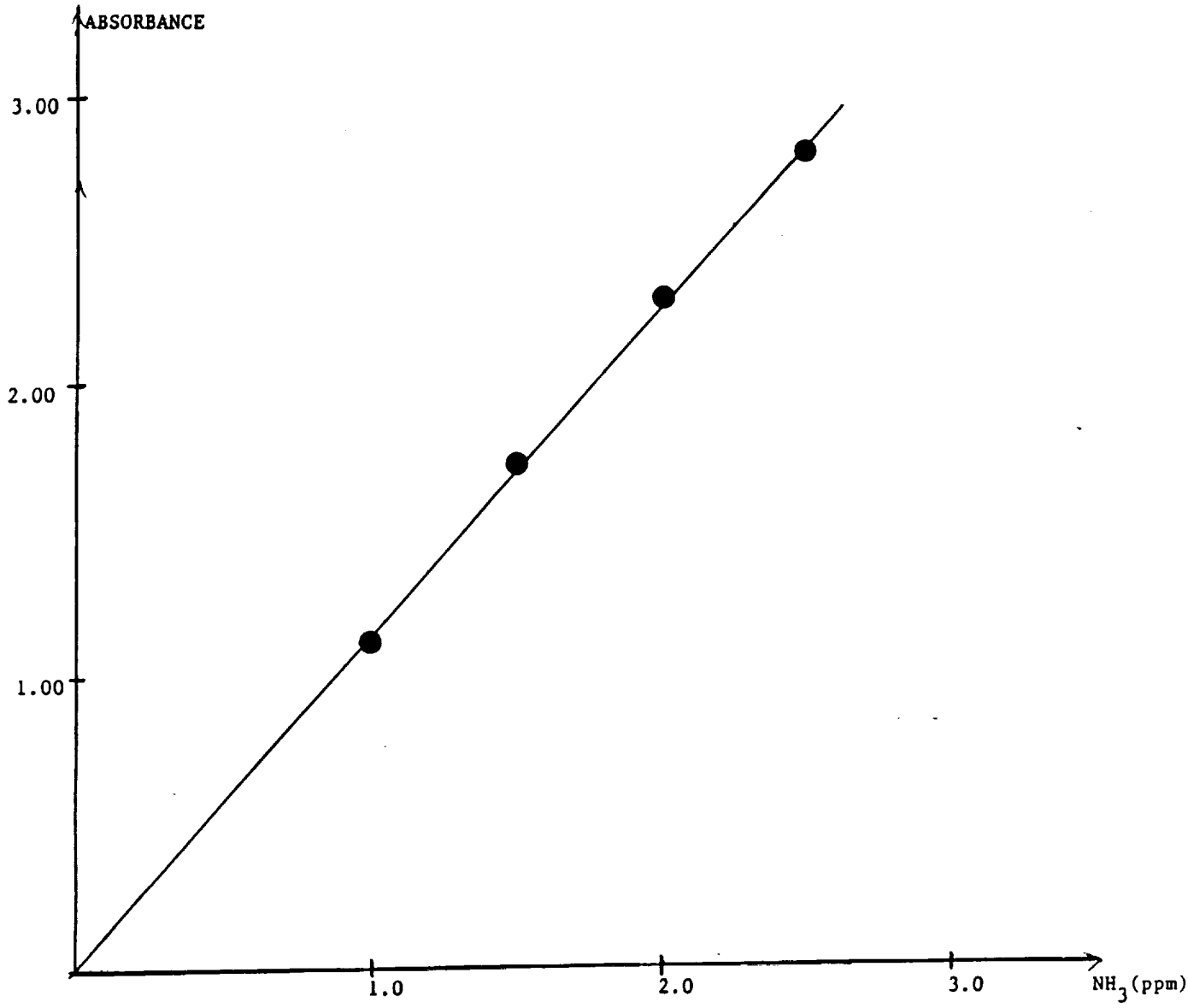


Figure 2 : Calibration of Absorbance with respect to ammonia concentration

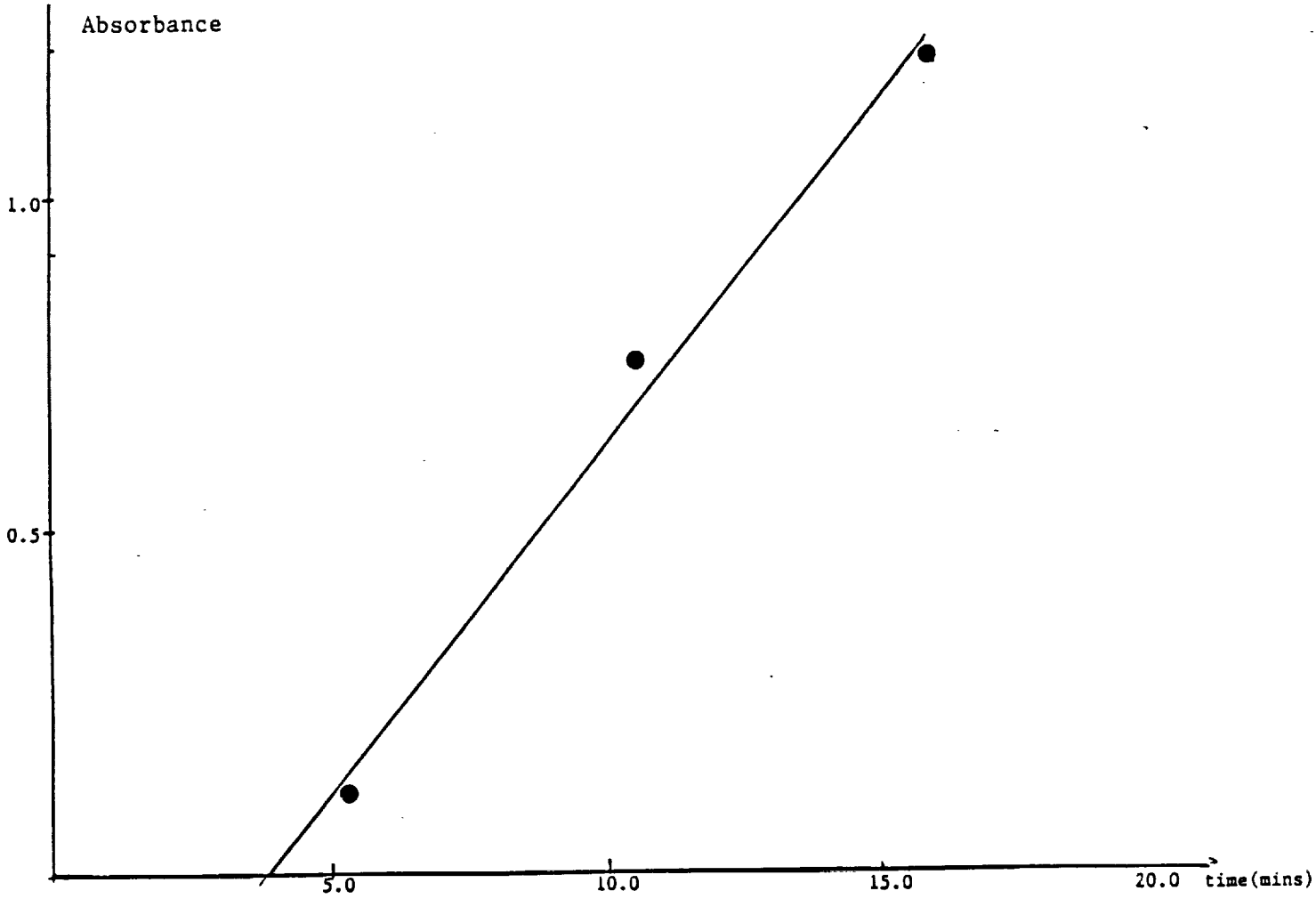


Figure 3: Activity of urease Vs. time

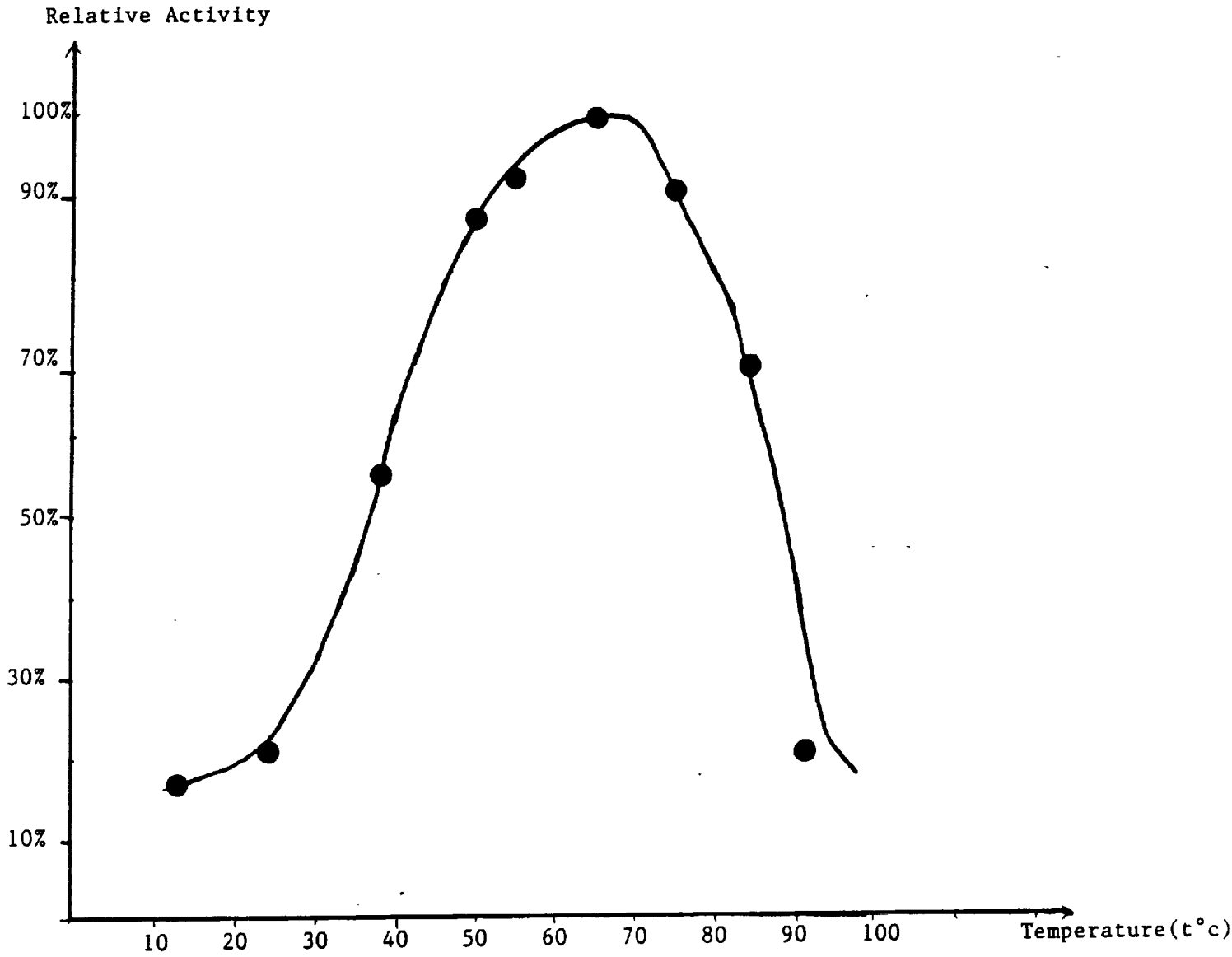


Figure 4: Activity of urease with respect to temperature

$1/T(10^{-3}K^{-1})$	3.50	3.37	3.22	3.10	3.05	2.96
log(V)	1.21	1.33	1.74	1.94	1.97	2.00

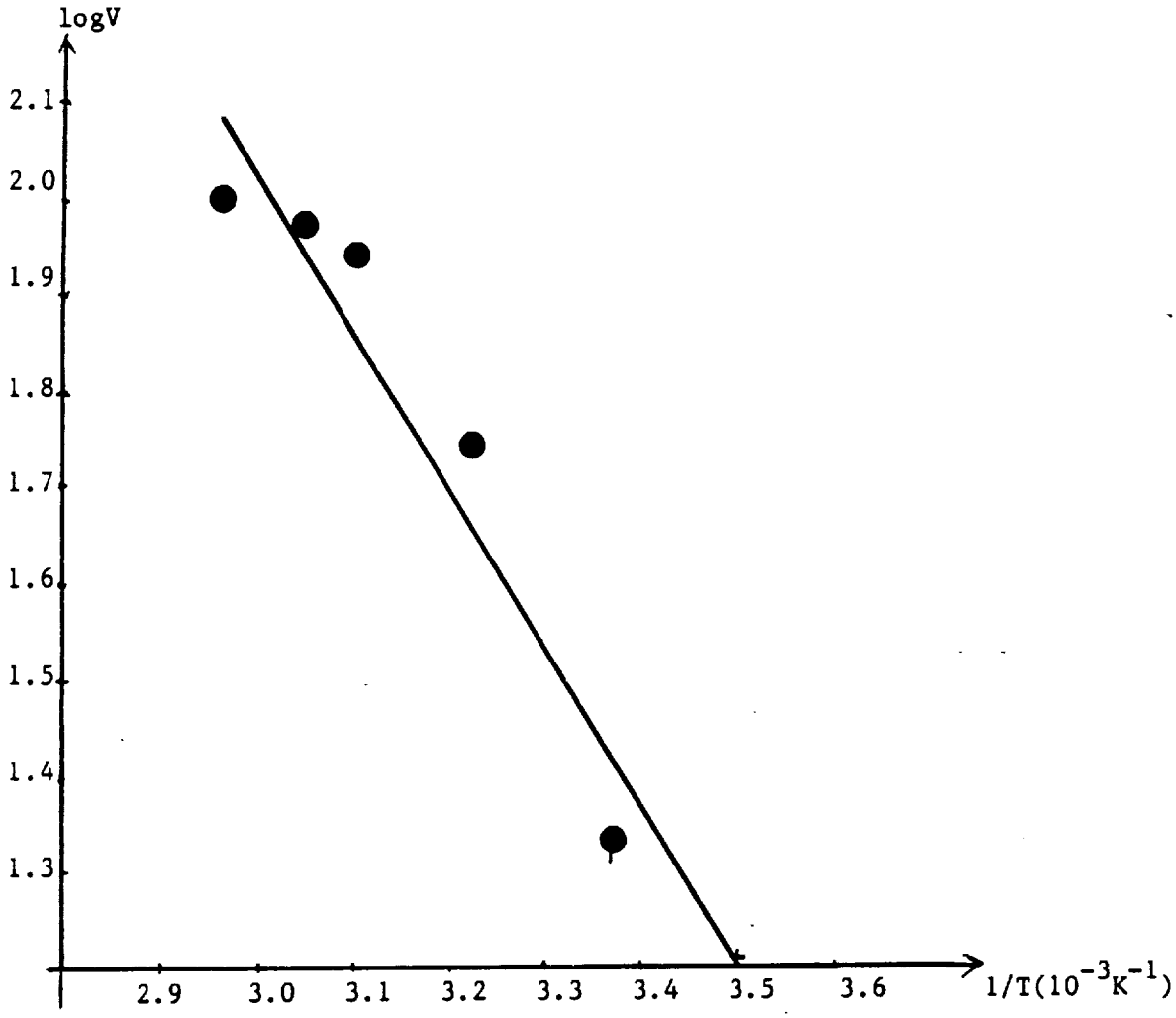


figure 5 : logV Vs 1/T

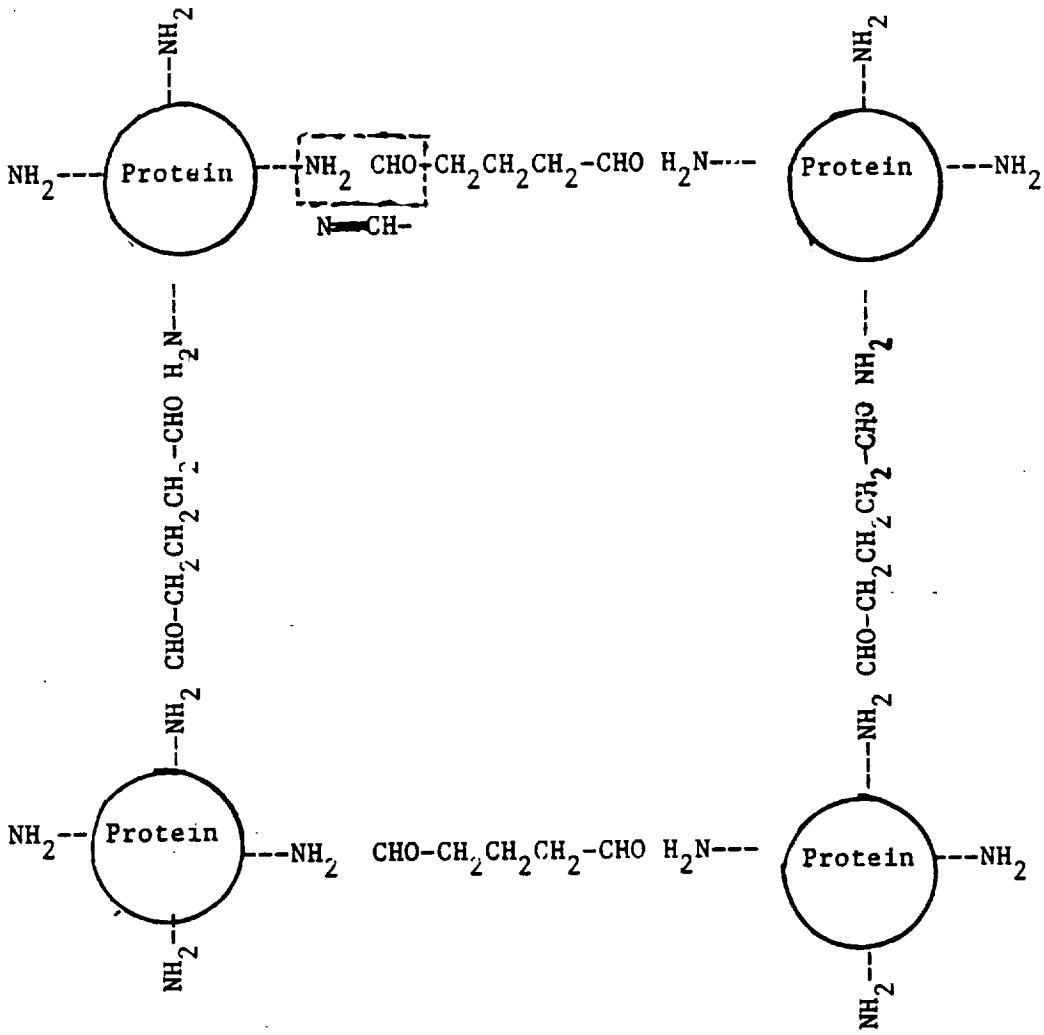


Figure 6 : Cross-linking protein by glutaraldehyde

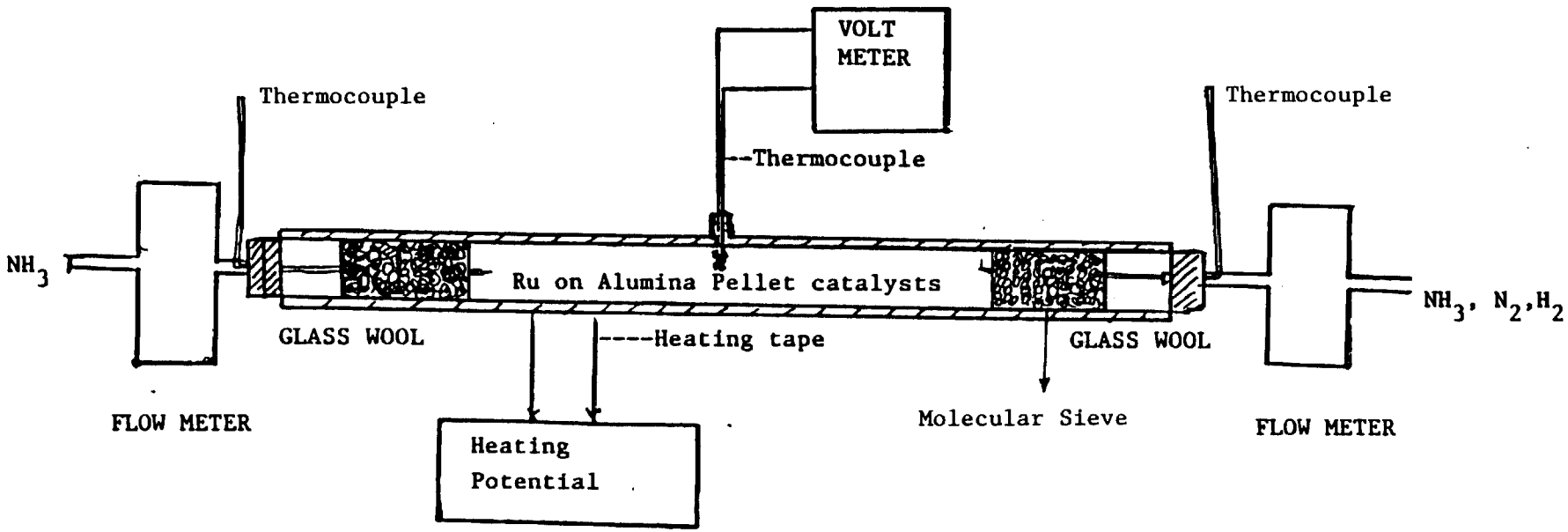


Figure 7 ammonia decomposition reactor

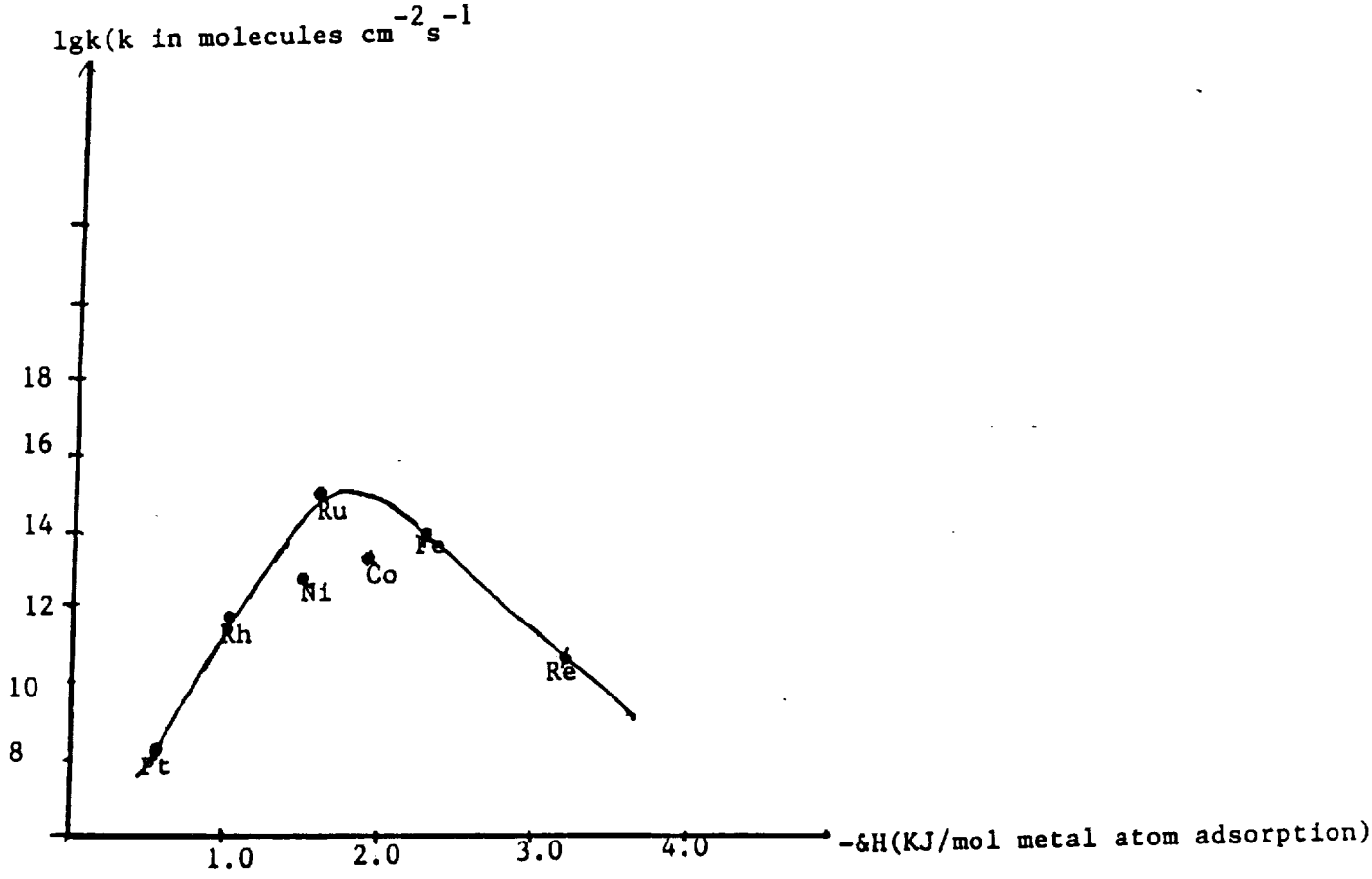
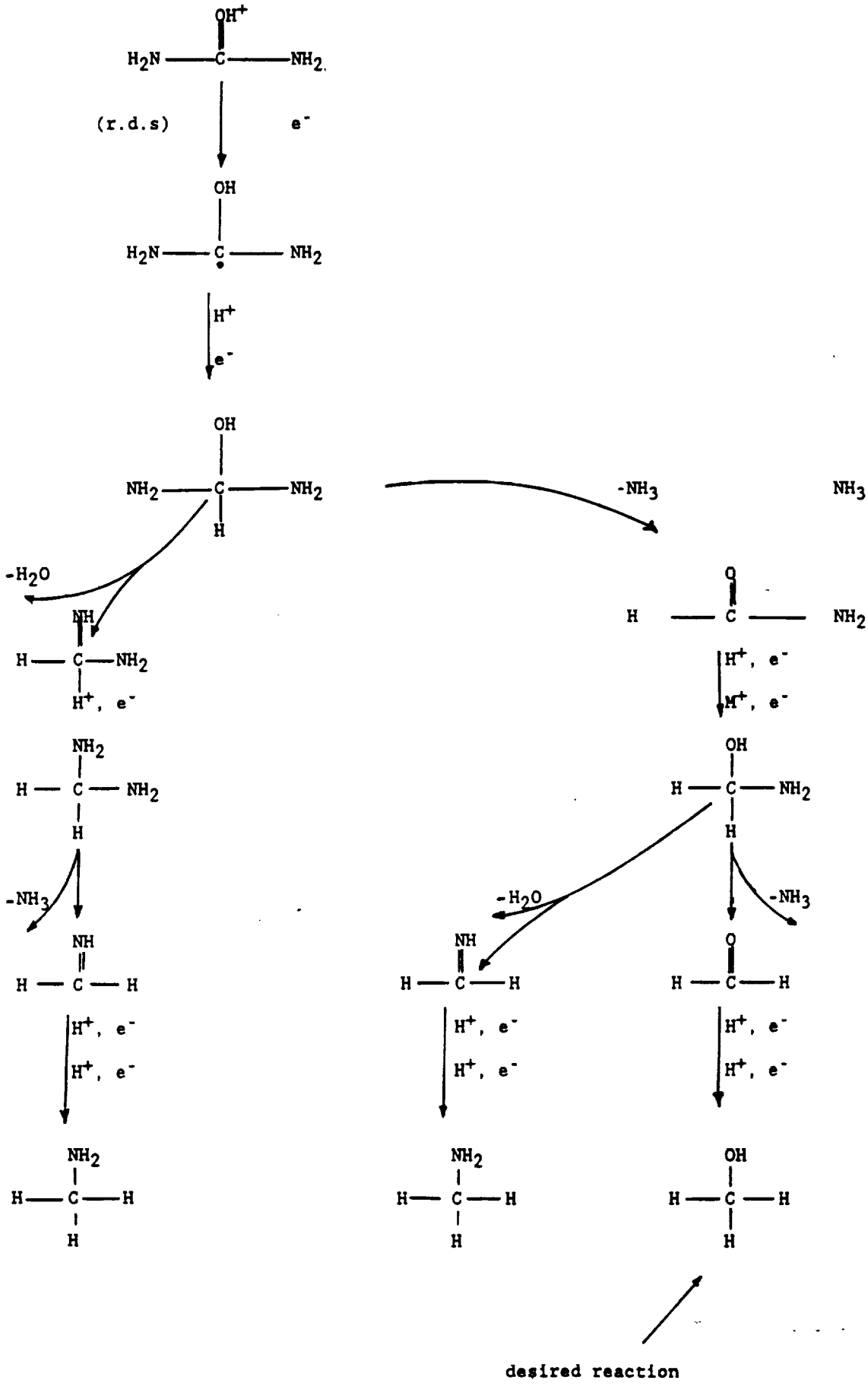


figure 8: The rate constant of different catalysts of NH_3 decomposition at t=400 c (from reference 1)

Figure 9 POSSIBLE MECHANISM OF UREA REDUCTION



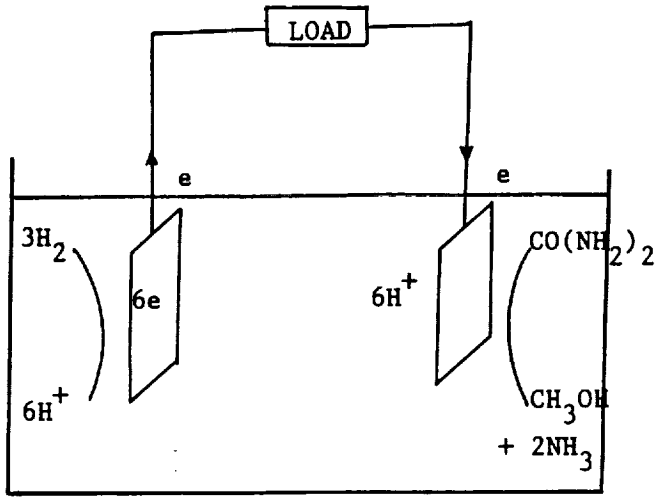


Figure 10: Fuel Cell Model for Urea - H_2 System.

Anodic Reaction: $3H_2 \rightarrow 6H^+ + 6e^-$ $E^o = 0.00V$
Cathodic Reaction: $CO(NH_2)_2 + 6H^+ + 6e^- \rightarrow 2NH_3 + CH_3OH$ $E^o = 0.02\text{-}0.00V$

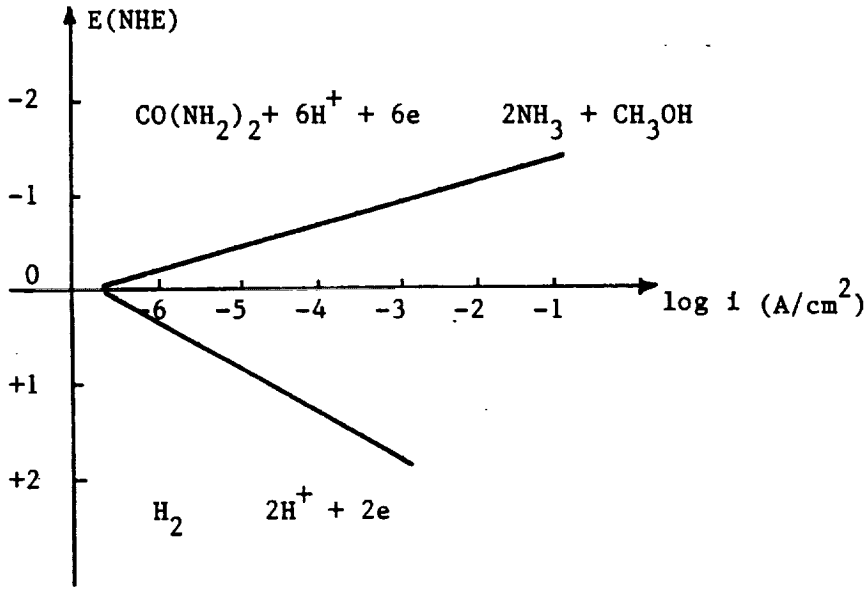


Figure 11