Effect of addition of NaCl on the functional properties of BMJ24 Jeewanu prepared under anoxygenic conditions (PUAC) in the water and in phosphate buffer of pH 6, 7 and 8 under anoxygenic conditions.

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Abstract— The present form of cell as we see it today has undergone evolution of 3.2 billions of years. According to Bahadur and Ranganayaki the earliest living cells was very simple in structure and were full of the properties of biological order. Bahadur in 1967 suggested that the way in which the systems organize themselves with properties of biological order is very important feature of the study of origin of life. Jeewanu, the autopoetic eukaryotes were prepared by Bahadur in 1967.

It was observed that when a mixture of Jeewanu and water is exposed to sunlight, water is split up into H_2 and O_2.But due to the nitrogenase like activity of Jeewanu, the produced hydrogen is utilized in the fixation of nitrogen. The hydrogen produced by the splitting of water is also utilized in the reduction of inorganic carbon. Khare prepared boron molybdenum Jeewanu in 1989 which showed significant water splitting. NaCl is a common constituent of the living cells.

An attempt has been made in this paper to study the effect of NaCl addition to the PEM on the functional properties of BMJ24 Jeewanu prepared under anoxygenic conditions (PUAC) in water and in phosphate buffer of pH 6, 7 and 8 under oxygenic conditions.

Keywords— Jeewanu, pH, BMJ24, PEM, NaCl, autopoetic, eukaryotes, exposure, Mineral solution, sunlight, PUAC, phosphate buffer, anoxygenic condition, pyrogallol.

INTRODUCTION

The present form of cell as we see it today has undergone evolution of 3.2 billions of years. According to Bahadur and Ranganayaki the earliest living cells was very simple in structure and were full of the properties of biological order. (Bahadur, K and Ranganayaki, S, 1964). [1] To tackle the problem of origin of life, it is essential to know how this earliest cells were synthesized under natural conditions. The molecular or the Chemical Evolution Theory given by Haldane (1929) [2] and Oparin (1924) [3] is the basis of the modern approach to the problem of 'Origin of life'. Bahadur in 1967 suggested that the way in which the systems organize themselves with properties of biological order is very important feature of the study of origin of life. (Bahadur, 1967). [4] The problem of origin of life in investigated basically in order to find out the natural condition under which the replicating, self-sustaining systems were produced. (Blum, 1961, Bahadur and Ranganayaki, 1966). [5,6]

The origin of life can be approached the best way if life and the living systems are considered in the light of functional properties. (Bahadur and Ranganayaki, 1980). [7]

It was observed that when a mixture of Jeewanu and water is exposed to sunlight, water is split up into H_2 and O_2.But due to the nitrogenase like activity of Jeewanu, the produced hydrogen is utilized in the fixation of nitrogen. Kumar in 1982 estimated the fixed nitrogen of the exposed mixture and thus confirmed the fixation of nitrogen by Jeewanu chemically. (Kumar, 1982). [8] The hydrogen produced by the splitting of water is also utilized in the reduction of inorganic carbon. (Smith et al, 1981). [9] They observed that on exposure to light from a mercury lamp, the aqueous mixture of Jeewanu, $NaHCO_3$ and water shows the appearance of ^{14}C in the organic material.

When the Jeewanu water mixture is kept in Warburg's flask, which is attached to the manometer filled with mercury and the entire apparatus is exposed to sunlight and kept in shade after some time, it is observed that the pressure in the flask is changed. Three reactions take place in the flasks: (i) splitting of water (ii) fixation of nitrogen (iii) loss of fixed nitrogen and the change in the pressure of the flasks might be due to the resultant of the gases evolved and fixed by these reactions. Reaction (i) is photochemical and stops as soon as light is cut off. Reaction (ii) and (iii) are ionic in nature in presence of molybdenum Jeewanu which has nitrogenase like material and takes place both in light and shade. Although third reaction is slower than the second reaction and takes place all the time during the nitrogen fixation but can be observed in shade only after all the hydrogen set free is utilized in the fixation of nitrogen and is stopped as the pressure of the Warburg's flask increases.

In light, Jeewanu splits water molecule to hydrogen and oxygen and this increases the pressure in the Warburg's flasks. The hydrogen produced is very active and combines with nitrogen of the air of the overhead space of the mixture of Warburg's flasks. In presence of molybdenum Jeewanu having nitrogenase like activity, the ionic reaction of combination of hydrogen and nitrogen takes place both in light and shade. The increase in pressure due to the water splitting is completely stopped in shade and so the combination of hydrogen and nitrogen is more prominently observed in shade.

When kept in shade, a decrease in the pressure of the flasks is observed. This can be explained in the following manner:

When nitrogen combines with hydrogen, it can do in three ways (i) one volume of nitrogen combines with three volumes of hydrogen to give ammonia, (ii) one volume of nitrogen combines with two volumes of hydrogen giving hydrazine or (iii) one volume of nitrogen combines with one volume of hydrogen to produce diimine.

1. $N_2 + 3H_2O \rightarrow 2NH_3 + 1.\frac{1}{2} O_2$

(Ammonia)

2. $N_2 + 2H_2O \rightarrow NH_2{-}NH_2 + O_2$

(Hydrazine)

3. $N_2 + H_20 \rightarrow NH{=}NH + \frac{1}{2} O_2$

(Diimine)

In these reactions, from one volume of nitrogen 1.1/2, 1 and ½ volume of oxygen will be produced. When ammonia is formed, there will be an increase of 1.1/2 volumes of gas in the mixture per one volume of nitrogen combined, and so an increase in pressure of the Warburg's flask will be observed. In the formation of hydrazine, as one volume of nitrogen combines with one volume of hydrogen leaving one volume of oxygen, there is no change in the volume of the gas in the flask and thus no change in the pressure of the flask will be observed. But when diimine is formed, there will be a decrease of ½ volume of gas in the overhead space of the flask, as only half volume of oxygen will be left. Thus decrease of pressure can be observed only if nitrogen combines with hydrogen to produce diimine when the hydrogen needed is coming by the splitting of water molecules.

Yogesh Khare (1989) prepared boron molybdenum Jeewanu and reported that addition of sodium borate in the PEM in which Jeewanu are prepared, decreased the size of the Jeewanu and increased their number. (Khare, Y, 1989). [10]

Sodium chloride is widely distributed in nature. About 2.8% sodium chloride is found dissolved in sea water and it is a common constituent of the living cells. Blood contains 350-550 mg of sodium chloride per 100 ml. sodium chloride regulates the osmotic pressure of the body fluids. It participates in a number of reactions resulting in the formation of HCl in the stomach. In the present section, the effect of addition of sodium chloride on the pH and the blue colour intensity caused by the formation of Mo^{4+} in the PEM of BMJ24 has been investigated.

Bahadur and Ranganayaki (1970) have observed that the organo- molybdenum microstructures are able to split water into hydrogen and oxygen in presence of sunlight and fix molecular nitrogen. [11]

It was observed that addition of sodium chloride in the PEM of BMJ24 Jeewanu, produced particles of larger size and the hydrogen ion formation increased with period of exposure. (Srivastava, D., 1991) [12].

Effect of variation in the concentration of mineral solution, formaldehyde and ammonium molybdate on pH and colour intensity of the PEM of 1.531211 SMJ38 Jeewanu before and after exposure to sunlight was studied by Srivastava, D. [13] - [17]

Effect of irradiation of 1.5312211SMJ29 Silicon Molybdenum Jeewanu PEM with clinical mercury lamp and sunlight on the morphology of the silicon molybdenum Jeewanu was studied by Srivastava. [18]

Effect of addition of Methanol and Ammonium Molybdate to(0+15):30:10:20:10:10 SMJ8 Jeewanu on the morphology, pH and colour intensity of the PEM of the Jeewanu both before and after Exposure to Sunlight up to a Total of 32 Hours was studied by Srivastava, D. [19,20]

Variation in the blue colour intensity and the pH of the PEM of 1.531211SMJ29 silicon molybdenum Jeewanu when the PEM is irradiated with clinical mercury lamp and sunlight" was studied by Srivastava, D. [21]

Srivastava, D., "Study of the effect of addition of Sodium Chloride on the pH and blue colour intensity of the PEM of the BMJ24 Jeewanu" was studied by Srivastava, D. [22]

Study of the effect of NaCl addition on the functional properties of BMJ24 (PUOC) in water and in phosphate buffer of pH 6, 7 and 8 under oxygenic conditions was studied by Srivastava, D. [23]

Study of the effect of NaCl addition to the PEM on the functional properties of BMJ24 Jeewanu prepared under oxygenic conditions (PUOC) in water and in phosphate buffer of pH 6, 7 and 8 under anoxygenic conditions was studied by Srivastava, D. [24]

Study of the effect of addition of NaCl on the functional properties of BMJ24 Jeewanu prepared under anoxygenic conditions (PUAC) in the water and in phosphate buffer of pH 6, 7 and 8 under oxygenic conditions was studied by Srivastava, D. [25]

This paper deals with the study of the effect of addition of NaCl on the functional properties of BMJ24 Jeewanu prepared under anoxygenic conditions (PUAC) in the water and in phosphate buffer of pH 6, 7 and 8 under anoxygenic conditions.

EXPERIMENTAL

The following solutions were prepared:

1) 4% (w/v) ammonium molybdate
2) 3% (w/v) diammonium hydrogen phosphate
3) **Mineral solution:** This was prepared by dissolving 20.0 mg each of potassium sulphate, calcium acetate, magnesium sulphate, zinc sulphate, manganese sulphate and 10.0 mg of copper sulphate in 80 ml of double distilled water. One salt was dissolved completely before adding another salt. In a separate test tube, 50 mg of ferrous sulphate was dissolved in 10.0 ml of distilled water. To this, 0.1 ml of 6NH_2SO_4 was added to avoid hydrolysis. Both solutions were mixed. The total volume of the mineral solution was made to 100 ml by adding distilled water.
4) 36% formaldehyde
5) 3% (w/v) sodium chloride solution,
6) 5% (w/v) sodium borate solution

Each solution, except formaldehyde, was sterilized in an autoclave at 15 lbs for 15 minutes.
Two clean, dry and sterilized corning conical flasks of 250 ml capacity were taken and labeled from 1and 2.
To each flask, 15 ml of ammonium molybdate, 30 ml of diammonium hydrogen phosphate, 10 ml mineral solution, 20 ml of 36% formaldehyde and 10 ml of sodium borate were added. Then, to flask 2, 10 ml sodium chloride was also added. The total volume of flak 1 was 85 ml and the total volume of the flask 2 was 95 ml.
In a test tube, alkaline pyrogallol solution was prepared by mixing 0.15 ml of 30% aqueous solution of sodium hydroxide and 0.15 ml of 20 % aqueous solution of pyrogallol. This pyrogallol solution was divided into two test tubes and one test tube was kept in each of the two flasks labeled 1 and 2 in a standing position taking care that the alkaline pyrogallol and the mixture of the flask do not get mixed up.

The flask number 1 and 2 were tightly plugged with rubber cork. Each flask was shaken carefully and exposed to sunlight for a total of 24 hours giving 4 hours exposure daily. In the mixture of flask 1 and 2 the Jeewanu were prepared under anoxygenic conditions (PUAC). After 24 hours of exposure, the contents of each flask was filtered, dried and weighed and the yield was recorded.

PROCEDURE:

Nine clean and dry Warburg's flasks of total volume 14.5 ml and bottom area 9.5 sq.cm. were taken and labeled as 1 to 9. Flask 1 was treated as control having only 5.0 ml distilled water. 0.3 ml distilled water was kept in the side lobe. Flask number 2, 3, 4 and 5 were filled with 20.0 mg of 1.531201 BMJ24 (PUAC) Jeewanu without NaCl in its PEM and 4.0 ml distilled water respectively. In the remaining flasks labeled 6, 7, 8 and 9, 20.0 mg of 1.531211 BMJ24 (PUAC) Jeewanu with NaCl in its PEM and 4.0 ml of distilled water was added.

In the side lobe of each flask 0.3 ml of distilled water was added. Then, in flask 2 and 6, 1 ml distilled water, in flask 3 and 7, 1 ml phosphate buffer solution of pH 6, in flask 4 and 8, 1 ml phosphate buffer solution of pH 7, in flask 5 and 9, 1 ml phosphate buffer solution of pH 8 were added respectively. These flasks were attached to their respective manometers filled with mercury. The whole apparatus was then exposed to sunlight for half an hour. The pressure changes of the Warburg's flasks were recorded after every five minutes. Then the whole apparatus was brought in shade for half an hour, readings were taken at every five minutes intervals. The whole process was repeated twice in a day.

The readings of the control flask having water alone were subtracted from each reading at that time to account for the pressure difference due to the change in the temperature.

Working under anoxygenic condition

Before the commencement of the observations under anoxygenic conditions, the oxygen in the overhead space of the Warburg flask was completely absorbed in pyrogallol and alkali mixture. For this, 0.3 ml alkaline pyrogallol was kept in the side lobe of the Warburg flask after keeping the necessary contents in the flask. The side lobes of the experimental flasks were covered with the black cloth of same size and same material. The same procedure was adopted for the control Warburg flask also to record the pressure due to temperature variation. The side lobes of the Warburg flasks were covered with black cloth to avoid the photochemical decomposition of alkaline pyrogallol.

The tap of the manometers were closed. The pressure in the Warburg flasks decreased as the oxygen in it was absorbed in the alkaline pyrogallol kept in the side lobe. When the pressure became constant, the outer tap of the manometer was opened to allow the pressure of the Warburg flask to come up to the atmospheric pressure. The air which came in consisted of oxygen too and this oxygen was again allowed to be absorbed.

The process of opening the tap and allowing the air to go into the flask to nullify the pressure decrease in the flask was repeated several times till the oxygen of the overhead space of the Warburg flask was absorbed and there was no decrease in the pressure of the flask even after allowing the mixture to stand overnight. This gave indication that there was no oxygen left in the overhead space of the mixture. The side lobes of all the Warburg flasks were covered with the same size of black cloth to avoid photochemical decomposition of alkaline pyrogallol solution and to provide similar condition in all the Warburg flasks. Only at this stage, the experiments were started.

Always a Warburg flask containing only corresponding amount of water with alkaline pyrogallol in the side lobe was kept as a control along with the experimental flasks. In the control flask, no Jeewanu sample was kept. This control was kept near the experimental flask and its readings were taken simultaneously along with the reading of the experimental mixture. The atmospheric pressure was recorded by a barometer during the experiment.

Preparation of alkaline pyrogallol

0.15 ml of 30% aqueous solution of sodium hydroxide and 0.15 ml of 20% aqueous solution of pyrogallol were mixed and thus the alkaline pyrogallol was prepared.

OBSERVATIONS

TABLE – 1

Effect of sodium chloride on the amount of solid material formed in the two PEM.

S.N.	Type of PEM of BMJ24	Amount of solid material formed in the PEM in g
1	PEM without NaCl (PUAC)	0.6890
2	PEM with NaCl (PUAC)	0.6390

It was observed that the amount of solid material formed in the PEM without NaCl (PUAC) was slightly more.
The readings of the Warburg's flasks were as follows:

TABLE – 2

Pressure changes in Warburg's flasks (in cm of mercury), containing 1.531201 BMJ24 (PUAC) without NaCl in the PEM and 1.531211 BMJ24 (PUAC) with NaCl in the PEM, in water and in phosphate buffer of pH values 6, 7 and 8 under anoxygenic conditions

Time in minutes	1.531201 BMJ24 (PUAC) without NaCl				1.531211 BMJ24 (PUAC) with NaCl			
	water	pH of phosphate buffer			water	pH of phosphate buffer		
		6	7	8		6	7	8
Sunlight								
5	−0.6	−1.3	+0.2	+0.5	−0.5	+1.0	+0.4	+0.7
10	−0.4	−0.8	+0.3	+0.2	−0.5	+0.5	+0.3	0.0
15	+0.2	−0.7	+0.9	+0.5	+0.1	−0.4	+0.9	−1.5
20	−0.4	−0.2	+0.5	+0.3	+1.0	+0.1	+0.5	−2.0
25	−0.2	−0.3	+0.4	+0.2	+0.1	0.0	0.0	−2.8
30	−1.5	−0.2	+0.5	+0.3	+1.2	+0.1	+0.4	−3.1
Shade								
5	−0.1	−0.7	−0.3	−0.5	−0.1	−0.4	−0.9	−3.6
10	+1.5	−0.8	−0.1	0.0	+1.7	−0.6	−0.9	−3.6
15	+1.2	−0.4	−0.4	−0.6	+1.1	−0.3	−0.9	−3.5
20	+1.7	−0.6	−0.8	−0.9	+1.6	−04	−1.2	−4.3
25	+1.8	−0.6	−0.9	−0.4	+1.7	−0.4	−1.2	−1.0
30	+1.7	−0.6	−1.0	−0.2	+1.7	−0.4	−1.1	−3.7
Sunlight								

5	+2.2	0.0	−0.6	−0.6	+2.0	−0.8	−0.6	+3.8
10	+2.0	−0.4	−0.1	−0.2	+1.9	−0.6	+0.1	−3.8
15	+2.5	−0.2	+0.4	+0.7	+2.2	−0.3	+0.6	−3.1
20	+2.3	−0.2	+0.8	+0.5	+2.1	−0.3	+0.5	−3.1
25	+2.4	−0.5	+0.8	+0.9	+2.2	−0.6	+0.3	−2.7
30	+3.8	−0.5	+0.9	+0.3	+3.5	−0.7	+1.8	−3.5
Shade								
5	+3.7	0.0	−0.6	−0.3	+3.6	−0.1	−0.9	−4.4
10	+3.2	−0.5	−0.8	−0.9	+3.2	−0.3	−1.1	−4.9
15	+3.1	−1.0	−0.9	−0.1	+3.1	−0.6	−1.0	−4.4
20	+3.3	−1.0	−0.7	−0.1	+3.1	−0.4	−0.9	−4.2
25	+3.1	−0.8	−1.0	−0.2	+2.8	−0.2	−1.0	−4.2
30	+3.0	−0.7	−1.2	−0.2	+2.7	−0.2	−1.3	−4.5

CONCLUSION

Under anoxygenic conditions 1.531211 BMJ24 Jeewanu (PUAC) produced in the PEM having NaCl showed slightly less water splitting and also slightly less nitrogen fixation as compared to 1.531201BMj24 Jeewanu (PUAC) without NaCl.

There was not much change in the trend of the reaction when phosphate buffer of pH 6 and 7 were added but on addition of phosphate buffer of pH 8, the mixture having 1.531211 BMJ24 Jeewanu (PUAC) produced in the PEM having NaCl showed maximum fixation of nitrogen.

Under oxygenic conditions 1.531211 BMJ24 Jeewanu (PUAC) produced in the PEM having NaCl showed better water splitting but under anoxygenic conditions, it showed better nitrogen fixation. The mixture having1.531201 BMJj24 Jeewanu (PUAC) produced in the PEM without NaCl showed better fixation of nitrogen in shade under oxygenic conditions and better water splitting in sunlight under anoxygenic conditions.

Thus, in general, the most efficient particle for water splitting was 1.531201 BMJ24 Jeewanu (PUAC) produced in the PEM without NaCl under anoxygenic conditions as indicated by the maximum increase of 3.8 cm in pressure and the particles showing best nitrogen fixation was 1.531211 BMJ24 Jeewanu (PUAC) prepared in the PEM having NaCl, in presence of phosphate buffer of pH 8 as indicated by the maximum decrease of 4.9 cm in pressure in Warburg's flasks.

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