Isolation of Ureolytic and Sulphate Reduction Bacteria: Acclimitize to Concrete Environment

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***Abstract*—**This paper presents on results of ureolytic and sulphate reduction bacteria isolated from fresh urine and acid mine water respectively. Over enrichment period both bacteria were enriched under alkaline and anaerobic condition to suit the concrete environment. Both enrichment media also were supplied with urea and sulphate salt for ureolytic and sulphate reduction bacteria respectively to ensure only the desired strain growth. The results showed that optimal growth condition of ureolytic bacteria and sulphate reduction bacteria are pH 10.71 and 9.35 respectively. It was observed that the gram stain was positive for both types with the shape of Coccus. It is hope that the isolated strains are able to sustain in concrete environment as bacteria concrete and could be used to improve concrete properties.

***Keywords*—**Bacteria concrete, ureolytic bacteria, sulphate reduction becteria,

I. Introduction

CONCRETE with high-performance are frequently used in construction practice, not only because of their high-strength qualities, but also because of their high resistance to other external influences. Therefore many studies are looking for a good quality of concrete which can improve concrete properties. There are various methods for improving concrete properties one of the methods by employing bacteria in concrete. Bacteria play an important role in bio-concrete promoting deterioration in porous materials, improve sand properties, sealing of concrete cracks to highly durable material and finally enhance the durability of building materials [1, 2]. Recently, mineral precipitation by microbial that resulting from metabolic activities of some microorganisms consider as area of researches in construction materials to improve the overall behavior of concrete.

These bacteria have the ability to precipitate calcium carbonate by the production of urease enzyme. However, the precipitation of calcium carbonate crystals occur by heterogeneous nucleation on bacteria cell walls once super-induced calcium carbonate precipitation has been proposed as an alternative and environmental friendly crack repair technique. Microbial carbonate precipitation decreases the permeation properties of the concrete. Hence, a deposition of a layer of calcium carbonate on the surface of concrete resulted in decrease of water absorption and porosity [3].

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Nevertheless, when bacteria are used to heal crack in concrete, the major hindering factor is the highly alkaline conditions of concrete that restricting the growth of bacteria. Therefore, it is necessary to isolate bacteria to suit high alkaline conditions. This study focuses on isolation of bacteria that can survive in the alkaline environment during isolation process, before used in the concrete mix. In this study, two type of bacteria are used namely, ureolytic and sulphate reduction bacteria which the source of bacteria were from fresh urine and fresh acid mine water collected from Sg pelepah Kota Tinggi Johor Malaysian respectively.

II. Factors Affecting Bacteria Growth

Bacteria cannot tolerate with all environment conditions because there are some factors restricting the growth of bacteria such as temperature, type of growth medium, extreme pH value and anaerobic conditions [4]. In this study two factors namely growth medium of bacteria and anaerobic conditions will be explored. Two type of bacteria were isolated namely, ureolytic and sulphate reduction bacteria (SRB) with different growth medium. This bacteria was enriched with specific growth medium, ureolytic bacteria was supplied with 10% of 40% Urea. For the case of sulphate reduction bacteria (SRB) few sulphate sources were tried to ensure that the sulphate fully dissolved in water and resulted with high pH value was choosed. In this study namely magnesium sulphate heptahydrate AR (MgSO4.7H2O), sodium sulphate (NaSO4), Zinc sulphate, AR (Zn SO4.7H2O), Magnesium sulphate 1hydrate (MgSO4.H2O) were tried. Nevertheless, magnesium sulphate heptahydrate AR (MgSO4.7H2O) was selected as appropriate source of sulphate for (SRB). The Magnesium sulphate heptahydrate AR (MgSO4.7H2O) had resulted to a high value of the desired pH when adjusted with sodium hydroxide.

III. Materials And Methods

A. Isolation And Characterized Of Bacteria Strains.

The source of ureolytic bacteria is from fresh urine while (SRB) was collected from acid mire water from Sg Pelepah Kota Tinggi, Johor Malaysia. The sample of (SRB) was taken over the dry season to avoid auto dilution of sample. The sample was collected from the discharge point of the mining retention pond. The sample collected was kept in a PTFE bottle and kept in a box with ice cube to ensure the condition of the sample remain stable before transport to the lab. Upon

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arrived the lab the sample was kept at 4^0C until enrichment process. Isolation process of ureolytic and sulphate reduction bacteria (SRB) were conducted following process of enrichment, serial dilution streaking plate, strain purification and gram staining. All process and media used were autoclave at 121^0C for 15 minutes for sterilization.

B. Enrichment

Enrichment process was done for two types of bacteria sulphate reduction and ureolytic bacteria. The compositions of media for both types are as follow.

(1a) Composition of sulphate reduction bacteria sample (SRB) consists of 25 ml (nutrient broth) + 10 ml (Mg $SO_4.7H_2O$) + 1 ml (water from Sg pelepah Kota Tinggi).

(1b) Composition of control sample for sulphate reduction bacteria consist of 25 ml (nutrient broth) + 10 ml (Mg $SO_4.7H_2O$).

(11a) Composition of ureolytic bacteria consist of 25 ml (nutrient broth) + 10ml (urea 40%) + 1 ml (urine).

(11b) Composition of control sample for ureolytic bacteria consist of 25 ml (nutrient broth) + 10ml (urea 40%).

Bacteria can't tolerate with extreme pH value. Under acidic condition some bacteria cell will hydrolyze or enzyme inactivate. The pH value plays an important role in microbial life that will influence the dissociation and solubility of many molecules that indirectly influence microorganism [5,6]. Therefore, after preparation the samples pH was adjusted for each of the sample to be in alkaline condition. The pH adjustment was done using NaOH until pH value in the range of 9-11. The other condition that was monitored along this study is anaerobic condition of the enrichment. Nitrogen gas was purged in the enrichment flask before enrichment started. The concentration of oxygen was measured with dissolve oxygen meter with 0 ppm of oxygen in the flask. The flask was shaked at room temperature. The adjustment of pH and anaerobic condition were done every day over the enrichment period of 40 days. The set-up of enrichment flask as shown in Fig.1

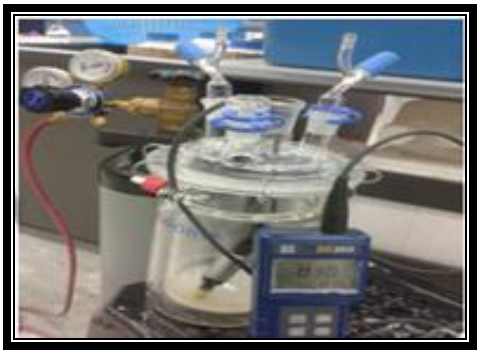


Fig.1 Enrichment flask

C. Dilution and Pure Streaking Plate Method

Along 40 days of enrichment the flask was turned turbid. Every 10 days 0.1 ml of the culture was taken and added with 9.9 ml of sterile distilled water. The serial dilution process as was done to both samples. Yeast extract and sulphate API agar were prepared for Ureolytic and (SRB) respectively. Each of the dilution was aseptically plated on yeast extract and sulphate API agar which were appropriately labeled. The plates were incubated at 30^0C in incubator until the desired strain growth. Colonies appeared were picked up and cultured. Fig. 2 illustrates agar with colonies of bacteria.

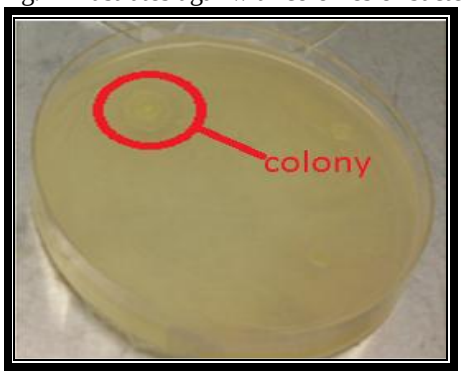


Fig.2 Plate with colony

D. Strain Purification

Purification step were performed until pure strain was obtained. Purification involve streak plate method was done to ensure only single strain obtained in each plating.

E. Gram Staining and Microscopic

Gram staining is used to investigate the major categories of microorganism or examine the morphology. In this study simple stains including crystal violet, iodine and safranin were applied. Two groups of bacteria can be distinguished with gram staining. Using this method, gram positive bacteria cell wall will retain crystal violet while gram negative change to red color.

IV. Result And Discussion

To identify the right source of sulphate as growth medium for sulphate reduction bacteria, four types of sulphate were tested namely, Sodium sulphate ($NaSO_4$), Zinc sulphate AR ($ZnSO_4.7H_2O$), Magnesium sulphate1hydate ($MgSO_4.H_2O$), and Magnesium sulphate heptahydrate AR ($MgSO_4.7H_2O$). The sulphates were tested according to changed of alkalinity after addition of NaOH. Table 1 presents the pH value for each type of sulphate salts. $ZnSO_4.7H_2O$ and $MgSO_4.H_2O$ sulphate resulted to low value of pH compare to $NaSO_4$ and $MgSO_4.7H_2O$. Even though $NaSO_4$ has the highest pH value but hardly dissolve in water. Therefore, $MgSO_4.7H_2O$ is chosen based on the criteria to be dissolved in water and resulted to alkaline pH.

The pH and anaerobic condition were controlled along the enrichment process. At initial stage of enrichment it is observed that it is quite hard to maintain the pH value to achieve 9 to 11 as shown in Table 2. The Sulphate reduction bacteria requires more drops of NaOH. On the other hand, ureolytic bacteria only used high drops of NaOH at the first day

TABLE I
SHOWN "PH'' VALUE FOR EACH TYPE OF CHEMECHAL SULPHATE

No	Type of sulphate	The value of PH with different drops of NaOH					
		0	10	20	30	40	50
1	Sodium sulphate ($NaSO_4$)	6.21	11.09	11.47	11.70	11.82	11.88
2	Zinc sulphate AR ($ZnSO_4.7H_2O$)	4.17	5.08	5.31	5.31	5.11	5.10
3	Magnesium sulphate1hydate ($MgSO_4.H_2O$)	6.62	7.11	7.25	7.38	7.42	7.38
4	Magnesium sulphate heptahydrate AR ($MgSO_4.7H_2O$)	6.88	8.16	8.44	8.48	8.62	8.66

TABLE II
SHOWN THE NUBER OF NAOH DRUPS AND THE VALUES OF "PH" FOR EACH SAMPLE

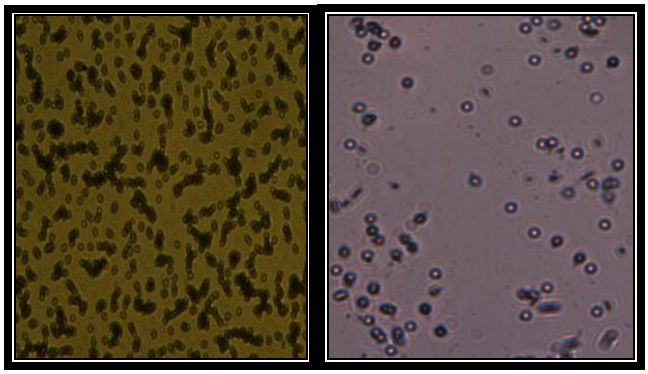
Type / Days	Sulphate reduction bacteria		Ureolytic bacteria	
	NaOH 1m drops No.	PH Value	NaOH 1m drops No.	PH Value
1	40	9.04	40	9.04
2	20	9.06	10	9.22
3	30	9.0	0	9.01
4	10	9.35	10	10.71
5	20	9.05	0	10.15
6	10	8.97	0	9.85
7	20	9.09	0	9.57
8	10	9.02	0	9.23
9	10	8.89	0	8.86
10	10	8.99	10	9.17
Total	180		70	

The results showed that bacteria capable to grow in alkaline environment and anaerobic condition. Two distinct colors such as cream and yellow were observed for all colons during purification process. The cultured colonies formed in the isolation medium with diameters range from 0.2mm to 1mm. The study observed that colonies were smooth and they had moist surface. As shown in Table 3 both types of bacteria ureolytic and sulphate reduction were in the shape of coccus with the period of enrichment of 10 and 20 days respectively. Previous researches used bacteria in the concrete in the shape of bacillus.

The observation also showed that colony morphology for 10 and 20 days enrichment with gram stain was positive for both ureolytic and sulphate reduction bacteria. Therefore, bacteria had the thicker peptidoglycan wall and blue color as shown in Fig.3(a,b) In the term of population, bacteria for 10 days is more than 20 days of enrichment.

TABLE III
SHOWN THE CHARECTERISTIC FOR SULPHATE REDUCTION BACTERIA AND SULPHATE REDUCTION BACTERIA IN THE PERIOD OF 10 AND 20 DAYS.

Source of bacteria	**Enrichment period**	**Colony color**	**Gram stain**	**Shape**	**Colony size**
Ureolytic bacteria	10 days	Yellow	+ ve	Coccus	In the range of 0.2mm to 1mm each type
	20 days	Cream	+ ve	Cocci	
Sulphate reduction bacteria	10 days	Yellow	+ ve	Coccus	
	20 days	Cream	+ ve	Cocci	



(a) (b)

Fig.3 Bacteria picture for 10 (a) and 20 (b) days respectively.

V. CONCLUSION

In this study bacteria successfully isolated to survive in the alkaline environment with anaerobic conditions. The ureolytic and sulphate reduction bacteria strains are Coccus in shape with shiny surface. The colony color was yellow, cream and the diameter was between 0.2mm to 1mm.

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