Potential of carbonic anhydrase and urease bacteria for sequestration of CO_2 into aerated concrete

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Abstract. The present study aimed to investigate the potential of bacterial strains from cement kiln dust (CKD) to sequestrate atmospheric CO_2 into aerated concrete as a functional for carbonic anhydrase (CA) and urease enzymes. Five samples of CKD was collected from Cement Industries of Malaysia Berhad (CIMA). The most potent bacterial isolates were selected and adapted to grow in 5% of CO_2 and in bio-aerated concrete medium. CA enzyme was detected by using a solution of 1.8 g of p-NPA (p-nitrophenyl acetate) and 25 mg of ampicillin at 7-pH. The results of thioglycolate broth medium assay indicated that the bacterial isolates were facultative anaerobic. Furthermore, the results of candle jar test reflected that the bacterial isolates have the ability to survive with 5% of CO_2 concentrations. Two bacterial isolates distinctly grow in bio-aerated concrete simulation medium, while only one bacterial isolate was the most potent and has produced in a powder form using freeze dryer to be ready to apply in bio-aerated concrete.

1 Introduction

The concentration of CO_2 rapidly increased worldwide especially in last five decades. The emission of CO_2 has increased from 300 ppm on 1950s to 400 ppm on 2010 as a result of anthropogenic activities [1-3]. Consequently, the increase of CO_2 level in atmospheric and other gasses emission led to catastrophic environmental phenomena such as, global warming, rise of sea level and climatic change [4]. Therefore, the technologies on CO_2 sequestration aimed to improve worldwide using different techniques such as; geological

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sequestration, chemical absorption, physical separation and biological sequestration [5-7]. Moreover, most of the researchers in this area are interested on natural sequestration as biological sequestration.

Biological sequestration taking place via algae, which help on conversion of CO_2 to calcite in the form of calcium carbonate ($CaCO_3$) or magnesium carbonate ($MgCO_3$)[8]. However, the use of algae in this applications facing challenge due to the size of photo-bioreactor required for algal cultures [8]. Nonetheless, different studies shifted to use bacterial cells, which have the ability to produce carbonic anhydrase (CA) enzyme as alternative technique to sequestrate CO_2 [9,10].

The application of urease producing bacteria in the concrete to improve its properties and self-healing purposes has been investigated by the researchers in the literature [11,12]. The reaction of urease in the bio-concrete contribute in the acceleration of CO_2 sequestration via chemical reaction, which functionally increase of $CaCO_3$ precipitation [13]. Nevertheless, one of the main challenges in the utilization of bacteria in the bio-concrete technology is the ability of the bacteria to survive in the concrete due to extreme pH and anaerobic conditions. Consequently, bacteria can be trained to adapt in high alkaline medium during the enrichment process or may isolated from inorganic materials which have extreme pH such as cementation materials [13-15]. The bacteria that candidate to use in the soil or concrete for the purposes of self-healing or CO_2 sequestration should be capable to produce urease and CA enzymes [9,10,12].

The current work aimed to investigate the potential of bacterial isolates for CO_2 sequestrating and $CaCO_3$ precipitating in the aerated bio-concreate.

2 Materials and Methods

2.1 Collection of samples and isolation of bacteria

Berhad (CIMA) located at Bahau, Negri Sembilan The samples (500 g) were collected in plastic bag and Berhad (CIMA) located at Bahau, Negri Sembilan The samples (500 g) were collected in plastic bag and transferred to the laboratory inside plastic bottles and then transported to the laboratory. The bacterial isolates were The flow chart in Fig. 1 shows the steps used for isolating of the most potent bacterial isolate to be used in the recovered from the (CKD) samples using a direct isolation technique in new medium at room temperature 30 ºC for 48 to 96 h as described in previous work [15].

2.2 Producing of CA and urease enzymes

The assay of CA was carried out in a broth culture medium containing 1.8 g of p-NPA (p-nitrophenyl acetate) and 25 mg of ampicillin at pH 7. The changing of the medium color from light yellow to dense yellow was indicated to ability of bacteria to produce CA [9,17].

The potential of bacterial isolates produce urease enzyme was tested as described by Benson [16]. In this test, the bacterial isolated sub-cultured in motility indole urea (MIU) medium, which has phenol red as an indicator. The ability of the bacterial isolates to produce urease lead to release NH_4 and then increased pH to alkaline; as a result the medium was changed to pink color.

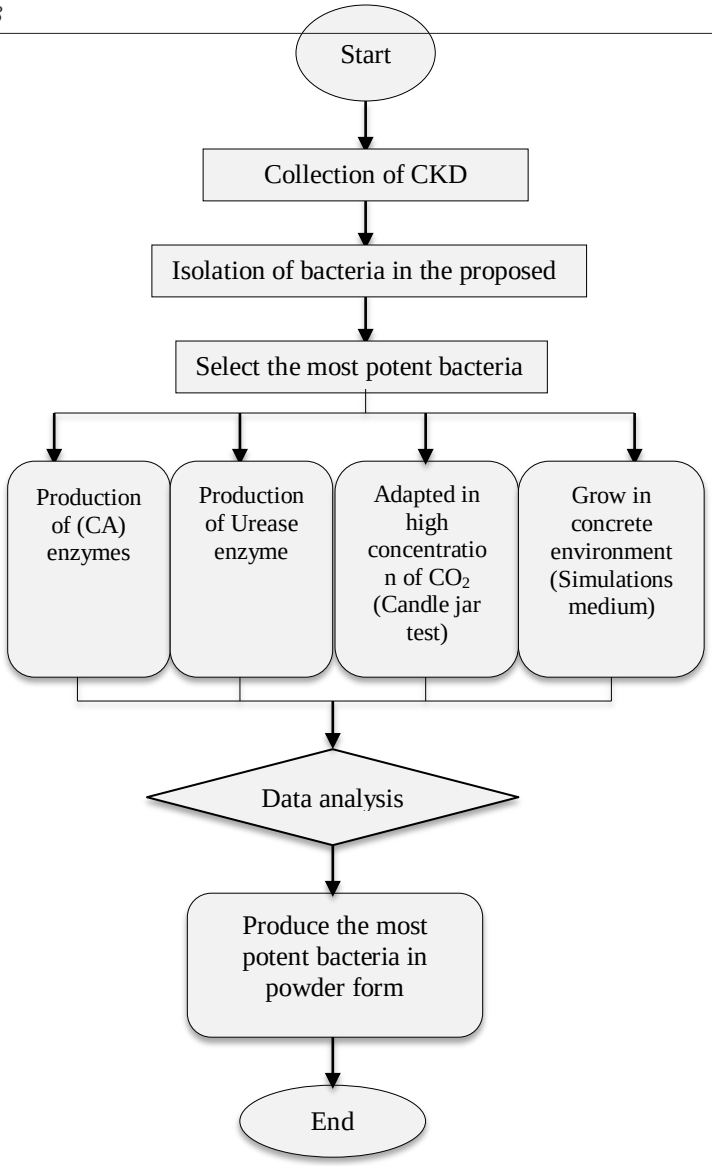


Fig.1. Methodology flow chart.

2.3 Thioglycolate broth assay

The bacterial isolates were sub-cultured in a thioglycolate broth medium to detect the favorable condition whether it's aerobic, anaerobic and facultative [16]. The bacterial growth at the top layer of the broth medium indicated for aerobic condition, which in need to oxygen. The growth of bacteria at the lower layer of the tube confirm that the bacteria are an anaerobic. The facultative bacteria has the ability to grow along the tube and nearly to bottom of the tube [16].

2.4 Adaptation of bacteria in CO_2 environment

The candle jar assay was conducted in this study to make sure that the isolated bacteria has the ability to grow with low level of oxygen and high level of CO_2. Therefore, each bacterial isolate was subculture in a new medium, the plates were placed in glass desiccator jar. Thereafter, a candle was placed and lit at the top on empty petri dish, then the glass desiccator cover of the jar put and immediately closed the stopcock as shown in Fig.2 [18]. The candle flame decreased after close the jar within approximately thirty seconds due to the depleting of oxygen, on another hand the level of CO_2 will increase to reach approximately 5% then placed in incubator at 37 °C. Every 24 h the plates smoothly take out from candle jar to check the growth of the bacteria colonies and relit the candle in jar and closed it to make sure the level of CO_2 constant [19-20].

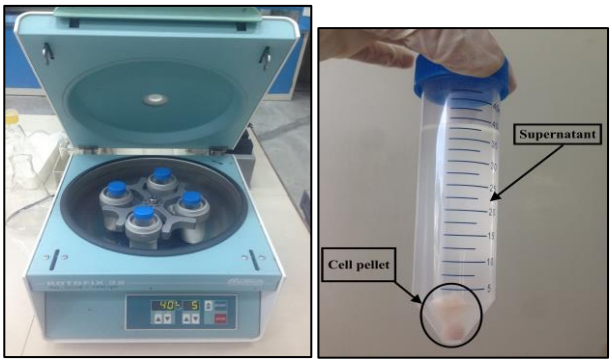
Fig. 2. Candle jar test

2.5 Simulation of bio-aerated concrete environment

Bacteria were applied in special medium to simulate bio-aerated concrete environments as follow; the proposed medium for isolation process + (1g/L) cement + (0.5g/L) fine aggregate + (0.05g/L) aluminum powder. One ml of the simulation medium cultured petri dish to investigate the growth and the concentration of bacteria cells daily [16].

2.6 Freeze drying of bacteria

Freeze drying used in this study was performed in order to produce bacteria cells in a powder form. The process was conducted for the most potent bacterial isolate. Bacteria cells were sub-cultured in a new broth medium at 30°C and shacked mechanically during the incubation time for 24 h. Then, immediately harvested by centrifugation at 4000 rpm for 5 minutes at normal temperature. The medium inside of centrifuge bottles was separating bacteria cell pellet drooped at the bottom of the tube and the medium supernatant Fig.3 [21]. Bacteria cells pellet were collected in autoclaved bottle and put into freeze-drying at -40°C and pressure less 0.133 mbar with no heat or energy supplied during the process along 96 h as shown in Fig. 4.



(a) (b)

Fig. 3. (a) Centrifuge machine (b) bacteria medium after centrifuge.

(a) (b)

Fig. 4. (a) Freeze drying machine (b) Bacteria in dry form inside the freeze drying machine.

3 Results and discussion

3.1 Isolation and characteristic strains

The alkalinities of the collected sample CKD were measured before isolation of bacteria, which in role gives prediction of the isolated bacteria, whether it have the capability to tolerate the extreme pH or not when they apply into aerated concrete.

The pH of the collected samples from area 1,2,3,4 and 5 were pH 11.82, 12.13, 12.12, 11.02 and 9.15 respectively. It can be seen clearly that, the pH values for all samples were in the range of 11 to 13, which almost reach range of concrete pH, except the pH value of sample that collect from area 5 wasn't attained concrete alkalinity [22]. The drop of pH of the sample that collected from area 5 may due to the weathering conditions at that area, whereas, the this area was directly exposed to the rain, which may cause regression in the pH value compared to other samples.

The results revealed that, the bacterial isolates grown slowly in the medium during enrichment process. Furthermore, the colonies of the bacterial isolates were growing in rough surface and cling strongly on the agar plates. Consequently, the features gives a high conjectured that the isolated bacteria colonies could cling strongly on concrete pores surfaces. The characteristics such as; color, size, shape and gram staining of isolated bacteria were different due characteristic and area of the collected samples [15].

3.2 Analysis of CA and urease enzymes activities

3.2.1 Production of CA enzyme

The ability of the bacterial isolates to produce CA enzyme was tested due to the important role of this enzyme in the CO_2 sequestration and formation of $CaCO_3$ [23]. The bacterial isolates were screened for CA enzyme activity in a broth medium (Fig 5). The results indicated that, CA enzyme produced successfully via all isolated bacteria [9,17].

The results demonstrating that, all isolated bacteria produced CA enzyme within two weeks clearly but in different degree in the color density. The highest production of CA enzyme was appeared clearly with bacterial isolate no. (401 and 501) respectively. However, bacteria no. (401) was produced CA enzyme more than bacteria no. (501). Meanwhile, the bacteria with no. (101, 201 and 301) were produced CA enzyme with low concentration.

The capability of the isolated bacteria to produce CA enzyme may interpreting by two reasons. The first reason, may refers to the high level of CO_2 in the environment that samples of bacteria (CKD) collected from, which may help the bacteria to produce CA enzyme and adopted in straggle conditions. The second reason, which agreed by previous studies [24] the bacteria isolated from inorganic materials gives a high potential to produce CA enzyme whereas, CA enzyme uses inorganic materials such as soil to handle CO_2 sequestration process [24].

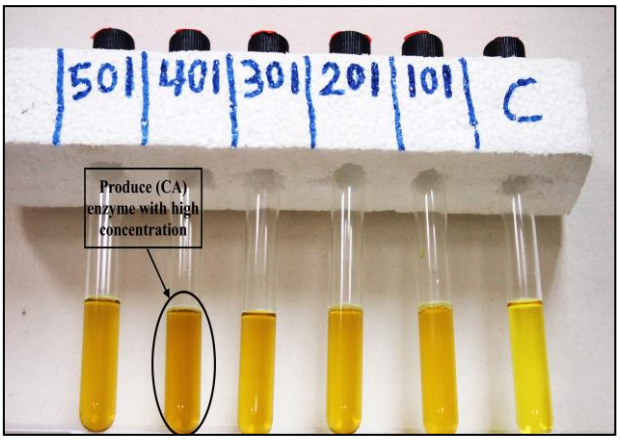


Fig. 5. Carbonic anhydrase (CA) enzymes assay at week two.

3.2.2 Production of urease enzyme

The results of urease enzyme production noted that the bacterial isolates were quite strange in order to produce urease enzyme. The color of all tubes did not change along the first five days. However, at day seven, bacteria with no. (201, 301 and 501) were starting to change the color to light pink which consider as positive results on producing urease enzyme, while bacteria with bacteria no. (101and 401) at the same period showed negative results because the color change to light yellow. However, the light pink colors in the tubes of bacteria no. (201, 301 and 501) bacteria did not sustained more than two weeks, which disappear quickly until they simulated near to the control sample. With continuous monitoring and evaluation it was observed that, bacteria with no.401 at day ten started to produce urease enzyme gradually and change the color from the light yellow slowly to pink color.

Consequently, the color totally changed two strong darkly pink at day 20, furthermore, the same color sustained more than three months at the same situation as shown in Fig.4. The performance of bacteria no. 401 can be interpreted that, this bacteria has the ability to produce CA and urease enzymes, however, it can produce CA enzyme faster than urease enzyme. Therefore, the colour at the first ten days changes to light yellow due to CA enzyme activities then change to pink when urease enzyme start produced in the tube of bacteria no.401.

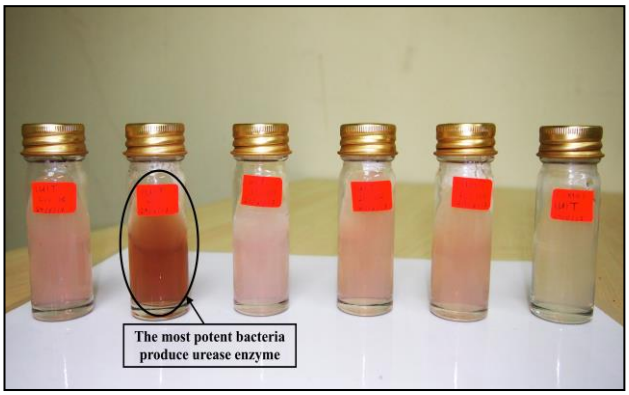


Fig. 6. The color of urease enzyme assay of the isolated bacteria after three weeks.

3.3 Aerobic or anaerobic condition via theioglycollate broth assay

The growth of bacteria cells in theioglycollate broth tubes response of oxygen atoms. Consequently, bacteria cells growth can be present in one of the three different situations namely aerobic, anaerobic and facultative anaerobe depend on the needed of bacteria to oxygen [25]. The aerobic bacteria cells have difficulties to survive without oxygen, therefore, they grow near to the top level of the tube because they are searching for oxygen. While, anaerobic bacteria cells grow at the bottom of the tubes because they are poisoned by high concentrations of oxygen. On other hand, facultative anaerobes have the ability to grow with or without oxygen so they grow in different levels of the test tube [25].

In this study the isolated bacteria grow clearly in the tubes contained theioglycollate broth. However, the results in Fig.7 demonstrate that, the growth of bacteria cells along the tubes, while the high concentration were nearly to lower level of the tubes. The growth way in theioglycollate broth emphasized that, all isolated bacteria have the ability to survive with and without of oxygen and they are under facultative situation [16].

The facultative situation gives a high potential of the isolated bacteria to adapt in aerated concrete due to the available of oxygen in its air babuls that already formed during mixture process [14,26]. Therefore, aerated concrete is the convenient concrete to apply the isolated bacteria in, because the extensive pores may help bacteria to practice its morphology activities normally and sequestrate high amounts of CO_2 compare to normal concrete.

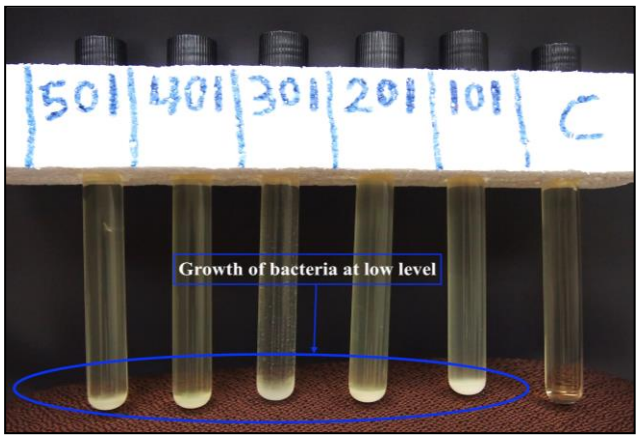


Fig. 7. Theioglycollate broth assay for isolated bacteria compare to control at 3 days.

3.4 Survival bacteria in candle jar

The used of candle jar test in this study not only as explorer test for the adaptation ability of isolated bacteria in the environment contained high level of CO_2, while it also can give a prediction results of producing CA enzyme which has the most important role on sequestration process of CO_2 [24].

The results demonstrated that, bacteria colonies presented in petri dish that contained the proposed medium within 24 hours in candle jar. Whereas, the growth of isolated bacteria in candle jar was faster than the growth of bacteria in the normal conditions inside the incubator. The colonies take around 24 hours to appear in petri dish, while in normal conditions growth of the colonies take three days. In addition to that, the growth of bacteria no. 401 was stupendous compare to other isolated bacteria, which present the high capability of this bacteria of adapted under high concentration of CO_2. The response of bacteria growth with available of CO_2 may has relation with producing of urease and CA enzymes; whereas, the growth of the bacteria those produce urease CA enzymes with high concentration grow fast and clearly in candle jar test.

3.5 Adoption of bacteria in bio-aerated concrete

All isolated bacteria applied in medium consisted of aerated concrete materials separately to simulate and investigate the capability of the bacteria to survive in the future environment of the bacteria inside bio-areated concrete.

The results were quite strange because the bacteria grow in the simulation environment of bio-aerated concrete were 301 and 401, however all the attempts those conducted to make sure of the adoption of bacteria with no, 101, 201 and 501 in the same medium were failed. Therefore, the candidate bacteria to be used in bio-concrete are the bacteria with no. 301and 401.

As mentioned in all previous sections under 3.0, the selection of the most potent bacteria subjected to serial tests not only to the simulation investigation of the bacteria into bio-aerated concrete, however, this test is the step that qualify the bacteria to transcend it from other in this competition. Therefore, the selection goes to the bacteria no. 401, because it the best among the others on producing urease and CA enzymes, adoption with CO_2, facultative and acclimatize in bio-aerated concrete environment as illustrate in Table 1.

Table 1. Selection of the most potent bacteria parameters.

Bact-eria No.	Parameters used to select the most potent bacteria in this study				
	Urease enzyme	CA enzyme	Facultat-ive condition	Survive in Candle jar test	Adoption in bi-aerated concrete
101	(+)	(+)	(++)	(++)	(-)
201	(+)	(+)	(++)	(++)	(-)
301	(+)	(+)	(++)	(++)	(+++)
401	(+++)	(+++)	(++)	(++)	(+++)
501	(+)	(++)	(++)	(++)	(-)

Note: the results descriptions in table as follow; (-) negative, (+) low (++) medium and (+++) high positive.

3.6 produce bacteria in powder form

The most potent bacteria produced in powder form using freeze-drying method successfully. After that, the powder bacteria were re-cultured in the proposed medium and investigate the concentration of bacteria in each gram using serial dilution process. The concentration of the bacteria in each gram was (3×10^{10}).

5 Conclusion

From the results and discussion it can be concluded that, the selection of CKD as bacteria sample was convenient to isolate bacteria capable to sequestrate CO_2 and adapted in bio-aerated concrete environment. Therefore, the isolated bacteria showed its ability to grow with high concentration of CO_2 and low level of oxygen. The efficiency of bacteria to grow in low oxygen confirm that its facultative bacteria. On the other hand, produce of CA and urease enzyme by isolated bacteria gives high potential to sequestrate CO_2 in aerated concrete. According to the tests conducted in this study the most potent bacteria was bacteria with no. 401, therefore, it's the candidate bacteria to produce in powder form and apply in aerated concrete in the future work.

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