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A Review on Sustainable Self-Healing Bacillus Bacterial Concrete on Water Absorption Test

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Abstract: Concrete is an important construction material as it can resist compressive loads. But if the load applied to the concrete exceeds its limit of resistance, the strength is reduced. Cracks might occur and treatment for cracks is quite expensive. To overcome this situation, introducing bacteria Bacillus strain mixed with concrete, in order to produce Calcium carbonate ($CaCO_3$) crystals form. This $CaCO_3$ can filled tiny cracks in the concrete and heal the concrete without any human intervention. The goal for this research is to provide an updated review on the effectiveness of Bacillus strain as concrete self-healing agent with different type Bacillus strain, concentration and growth medium by water absorption test. A systematic review is used to collect data from 9 journals and articles using Boolean Operators to combine or exclude keywords, which leads to more focused and productive results. Several databases were extensively searched, including Google Scholar and Science Direct. Through all the case study reviewed, the highest water absorption reduction is 70% with selection of Bacillus Pasteuri, cell concentration 10^6 cells/ml, and growth nutrient is NH4-YE medium which is 20g yeast extract and 10g Ammonium Sulfate $((NH_4)_2SO_4$. Other result resulting in water absorption reduction with range of lowest value 0% and the highest value is 60%. To ensure the Bacillus survive and give positive effect to the durability of concrete are the concentration and the growth medium of Bacillus strain. Most bacteria concentration can survive the alkaline conditions of concrete is 10^1 cells/ml to 10^{11} cells/ml. And most common growth medium that is effective for Bacillus to survive is 20g of yeast extract. It is proven that concrete embedded with bacteria has a lower water absorption capacity than nominal concrete.

Keywords: Concrete, Bacillus, Self-healing, Micro-cracks, Water Absorption Test

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1. Introduction

Concrete is one of the most widely used building materials globally. Concrete is in high demand because of its durability to withstand load and resistance to extreme weather conditions. However, if the force applied to the concrete exceeds its resistance limit, it loses strength and develops cracks, which are costly to repair. Plastic or hydraulic shrinkage and the formation of undesired micro and macro fractures all contributed to the low tensile strength of concrete [1]. As a result, the concrete structure's strength will deteriorate, and failure and collapse in the building structure might happen. Water can penetrate concrete and cause corrosion when it contacts the reinforcing steel of the structure. As a result, the concrete structure's strength will deteriorate, and failure and collapse in the building structure might happen. To overcome this situation, introducing bacteria from Bacillus strain to concrete produces calcium Carbonate ($CaCO_3$)form, plugging tiny fractures and holes in concrete. In previous study, researchers Mostafa Seifan [2] found bacillus strains that can grow in high alkaline environments. It is possible to use microbially induced $CaCO_3$precipitation for various purposes, including healing cracks and preventing Carbon Dioxide from entering the concrete, which will lead to corrosion [3].

The purpose of this study is to provide an updated review on the effectiveness of Bacillus strain as concrete self-healing agent with different type of Bacillus strain, concentration and growth medium by water absorption test in 28-days. Type of bacteria Bacillus strain investigated in this study is Bacillus Sphaericus, Bacillus subtilis, Bacillus magaterium, Bacillus Pasteuri, Bacillus colli, Bacillus flexus, Bacillus cereus, Bacillus Specie and Bacillus Anthracis. The material reviewed in this study is bio-concrete mixed with cement, fine aggregates, water and Bacillus strain. A systematic review is used to collect data from 9 journals and articles that has been chosen. From the 9 journal that has been reviewed, parameters between various types of Bacillus strain mixed in concrete with different types of concentration and growth medium was compared. All the concrete samples that added with Bacillus strain with different Bacillus strain, concentration and growth medium reviewed going through water absorption after 28 days of curing process.

2. Literature Review

Concrete is used to construct structures, a composite material primarily composed of Portland cement, water, and aggregates. When these components are combined, a mortar paste is formed, which will become concrete when it hardens over time. Concrete is so essential to our societies because it is the only construction material that can give the most negligible environmental impact for a structure or pavement throughout its entire lifespan at a reasonable cost [4]. Because concrete is weak in tension but strong in compression, cracks are inescapable in this material. When fractures appear in concrete, they can reduce the lifespan of the building. Water, air, and other potentially hazardous elements can enter through minuscule cracks. As a result, the concrete's ability to resist corrosion is reduced, becoming more susceptible to corrosion. If these fractures are not repaired immediately, the building may be at risk of collapsing entirely [5].

Self-healing concrete is a reasonable way to fix fractures in the concrete that can be done without the assistance of a professional. During the mixing process, bacteria and a calcium nutrient source are introduced to the concrete to improve its performance. Bacteria will accumulate in any fractures in the concrete, causing $CaCO_3$ to precipitate. Cracks bigger than 0.8mm in diameter are more challenging to repair. But cracks can be repaired with the help of bacteria and $CaCO_3$ precipitation in some cases [6]. To construct a self-healing mechanism in concrete, there are three basic ways that can be used. Which are autogenous healing, encapsulation of polymeric material and formation of microbial $CaCO_3$ [7]. As stated by Abhishek Thakur [8] microbiologically induced calcium carbonate precipitation (MICCP) by bacteria has been recognized as an environmentally friendly technology for improving the qualities of concrete.

Bacillus strains may be found in soil, fermented foods, air, and the human digestive system, among other locations. Bacillus strain is used in this review because of the durability or the bacteria to live in alkaline surroundings. Mostafa Seifan [4] in his research determined that the bacteria can survive under certain conditions where pH of cement and water is more than 13. When bacteria come into contact with bio-concrete, it is assumed that precipitation of $CaCO_3$ occurs. The urease enzyme, which is produced by Bacillus, will hydrolyze urea and generate carbonate ions. If there are too many calcium ions in the system, $CaCO_3$ will develop. Bacteria that generate large amount of urea hydrolysis can be catalyzed by bacterial urease. The decomposition of urea into ammonium and carbonate ions raises the pH from neutral to around 9. In the presence of nitrogen (Ca^{2+}), calcium carbonate ($CaCO_3$) may form. Cracks healing/sealing materials in bacteria-based self-heal systems are provided by biogenic $CaCO_3$. Depending on the amount and pace of urea decomposition, biogenic $CaCO_3$ production is directly linked to the effectiveness of fracture healing or sealing. The process is considerably faster if the amount of urease provided is sufficient, as indicated by Wang, Jianyun [9]. Yeast extract and urea were the primary ingredients in the growth media used by most Bacillus strains. The germination and spore growth was considerably more spectacular when yeast extract concentrations were between 5 and 20 g/L [9] Other types of growth media for bacterial culture may be uncovered in future case studies.

3. Methodology

As a type of research synthesis, systematic reviews are carried out by review groups that are highly skilled in identifying and retrieving relevant international evidence for a specific question or questions, as well as evaluating and synthesizing the findings of this search in order to inform practise, policy, and, in some cases further research. Unlike other types of research, systematic reviews follow a predetermined and planned strategy that necessitates the use of methodological techniques to guarantee that the findings are trustworthy and useful to end users [10].

Several databases were extensively searched, including Google Scholar and Science Direct. This research was conducted primarily using Mendeley. Mendeley is one platform that meets the information demands of researchers, educators, administrators, students, and librarians worldwide. Mendeley can generate bibliographies automatically and collaborate with other scholars online quickly and easily. 9 suitable articles was chosen based on the keywords of ‘Bacillus Strain’, ‘self-healing concrete’ and ‘water absorption test’.

The keywords were selected based on the topic which is the effect of Bacillus strain on concrete in water absorption test. The first step included identification based on the database using a Boolean operator to combine the terms which are “concrete” AND “self-healing” AND “fungus” AND “water absorption” . The result shows that concrete (n=574,000), self-healing (n=13100) and bacillus (n=5130), the total articles obtained based on these keywords were 5130. Then the article chosen based on year (2016-2021); this limiting factor has 4510 articles remaining. Then, the chosen articles were screened again manually based on the related title, which is 55 in total. The full article chosen is 55 and finally, number of case studies used in this review article were nine.

Table 1: Inclusion and Exclusion criteria

Criteria	Eligibility	Exclusion
Literature type	Research article Review article	Book, Conference paper, in press, news etc.
Language	English	Non-English
Time Line	2016-2021	<2016

Table 2: Keywords and searching strategy

Database	Keyword and search format
GOOGLE SCHOLAR	(TITLE-ABS-KEY (concrete)) AND ((self-healing)) AND (bacillus)

According to the study, articles and journals on this subject include concrete, bacillus, and self-healing. This study examines the effect of Bacillus strain as a self-healing agent on concrete and the factors that influence the self-healing ability of the Bacillus strain on bio-concrete. This study employs a qualitative analysis to aid in identifying data sources. Numerous studies on Bacillus strain as self-healing are considered to establish the most precise way for this subject. Google Scholar and Mendeley is the leading search engine that been used in this topic, and the keyword for this study is “concrete” AND “self-healing” AND “fungus” AND “water absorption” that obtain from a Boolean operator. Thus, the total reviewed articles used in this review article were nine been achieved.

4. Results and Discussion

There are 9 case study reviewed to investigate the effectiveness of various type of Bacillus strain mixed in concrete with different concentration and nutrient through water absorption test.

4.1 Effect and characteristic of different bacteria concrete on water absorption

According to research conducted by Abishek et al. [8], the addition of Bacillus cereus can increase compressive strength by up to 50% at a cell concentration of cells/ml, while the addition of Bacillus Sphaericus, Bacillus pasteurii, Bacillus subtilis, Bacillus flexus reduces water absorption by up to 81% to 88% after 28 days of curing. Water absorption was reduced in bacterial concrete by using the Bacillus strain in this research.

Research conducted by T. Shanmuga et al. [5], water absorption reduces as MBS and bacterial solution proportion increases. Calcium silica hydrate forms within the pores as a result of the MBS filling action on small holes and spaces in the concrete. Because of the spherical-shaped particles of MBS, water absorption increases as the MBS percentage climbs to 12%. Concrete infused with bacteria and 8 % MBS reduced water absorption by 15.46 % after 28 days than normal concrete. The lower water absorption of the concrete mix may be explained by the higher calcite formation in the MBS and bacteria-containing mix compared to the control mix. The results showed that bacteria might extend the service life of structures because of their high urease activity and ability to live longer than other bacteria.

Through research by Mingyue Wu et al. [14], Tthe water absorption of Bacillus cereus considerably reduced after bacterial treatment. Water permeability of specimens treated this way were 11% and 10.9% percent lower than normal concrete specimens, respectively. The results of bacterial crack healing have been quite promising.

As the result of experiment by Seshagiri Rao et al. [15], It is estimated that the water absorption capacity of bacterial concrete specimens is reduced by nearly 50–80% for low to high grade concretes when compared to the water absorption capacity of controlled concrete specimens. The absorption characteristics describe the volume and connectivity of pores in an indirect way.

Through experiment conducted by Nasrin et al. [16], water absorption was 5% for regular concrete specimens cured in the calcium lactate-urea solution, whereas it was 11.4 %, 3%, and 9.2 % for those reinforced with polypropylene, steel, and bar chip fibres, respectively. The significant decrease in water absorption found in bacteria-containing specimens may be attributed to urea and nutrition sources, which enabled the bacteria to produce calcite deposits deposited in concrete pores, reducing water absorption. It was shown that these sediments might reduce water absorption by as much as 50%. and 60% in comparison to the controls. Bacteria caused calcite deposition on the concrete surface, which prevented water from flowing through it. In both cases, surface-treated specimens healed in the calcium lactate-urea solution and the tap water exhibited a smaller reduction in water absorption than specimens cured in the calcium lactate-urea solution.

Another study by Arun Kumar et al., [17], Bacillus subtilis bacterial solution was directly added to the concrete mix, and calcined clay was used as a substitute for cement in varying proportions by weight of cement in the concrete specimen. Replacement of 10% calcined clay reduces water absorption value by 12.02 % after 28 days, respectively. 15 percent calcined clay replacement results in 8.75 % decline in water absorption value, whereas 20% calcined clay replacement results in 6.01 % drop in water absorption value. The water absorption capacity of bacteria-embedded calcined clay concrete is lower than that of nominal concrete and calcined clay concrete. Because of the usage of calcined clay as a filler component and bacterial metabolism, the thick and compact particles may be the reason of this.

In research by S. Yoosathaporn et al. [18], Water absorption of cement cubes made using CME-urea medium was 6.03% greater than that of the control, whereas water absorption of cement cubes prepared with Bacillus pasteurii grown in CME-urea medium was somewhat higher than that of the control. The voids of specimens generated with CME-urea media (38.35%) and those made with Bacillus pasteurii cultivated in CME-urea medium (3.20%) were 10.01% and 3.20 % greater, respectively, than the control.

Microbial concrete research by Sandip Mondal et al., [19] indicate the decrease in water absorption may be demonstrated to be directly proportional to the bacterial concentration. Interestingly, the rate of decrease of water absorption is practically the same for both bacteria during 28 days of testing, with water absorption reduced by 16%, 27%, and 36%, respectively, corresponding to bacterial cell densities of 10^3, 10^5, and 10^7 cells/ml of Bacillus subtilis.

Through the experiment result conducted by Saman Shahid et al. [20], The percentage of the water absorption increase after 28 days of curing process. This might occur because of the uncertain concentration of bacteria added into the concrete mixture. Another reason might be because of the nutrient used for growth of the concrete. Which is sodium alginate beads that is not common as yeast extract, beef, NaCl and etc as medium growth for the Bacillus strain bacteria.

4.2 Method producing bacteria

This study employs a qualitative analysis to aid in identifying data sources. Numerous studies on Bacillus strain as self-healing are considered to establish the most precise way for this subject.Previous studies have discovered that the process of making bacterial concrete requires several steps such as bacterial species selection, isolation, growth medium, test specimen preparation and characteristic of bacteria. According to prior research, there are several methods for incorporating microorganisms into concrete [8]:

i. Direct addition of microbiological broth or spores to new concrete. This approach of incorporating bacteria into concrete is easy, cost-effective, and results in more excellent biological concrete work-ability.

ii. On activated carbon or silica gel (Immobilised form). In this method, adherent microorganisms or spores are added to activated carbon or silica gel.

iii. Through encapsulation. This approach added encapsulated microorganisms straight into concrete.

iv. Using the vascular system. This approach results in the circulation of microorganisms within the concrete via the microvessels.

4.3 Type of bacteria strain

Type of bacteria strain reviewed is Bacillus. According to past study, some bacteria are harmful to human health in various ways. Another group of bacteria, such as Bacillus sphaericus, Bacillus pasteurii, Bacillus subtilis, and Bacillus flexus and other types of Bacillus, have no adverse effect on human health but do have a higher capacity for calcite precipitation, making them ideal bacteria for the design of bacterial concrete. To ensure the bacteria survive and present after mixing with concrete, the following test usually conducted by researchers:

I. Scanning Electron Microscopy (SEM) & Energy Dispersive X-Ray Analysis (EDX). SEM provides complete high-resolution images of Bacteria by rastering a focussed electron beam across the surface and collecting secondary or backscattered electron signals from the surface. An Energy Dispersive X-Ray Analyzer (EDX or EDA) offers information on the elemental composition of bacteria as well as the quantitative composition of the bacteria. [11]

II. X-ray diffraction (XRD). XRD common used to determine qualitative and quantitative crystalline phase analyses. It identify diffraction patterns or using special settings, such as characterization of solid solutions, crystallite size and shape or internal elastic strains, orientation and stresses at various levels, temperature effect [12]. It will specify the measurement of cementitious materials.

4.4 Result Comparison between Species of bacteria, Concentration, Growth Medium and Water Absorption Result

This is the summary of each element used for the systematic article review. The content in this table is the comparison between species of bacteria, concentration of bacteria, growth medium and water absorption result 28 days curing difference than normal concrete percentage: shown in [Table 3]

Table 3: Element used for each article

No	Author	Species of bacteria (Bacillus)	Concentration of Bacteria	Growth Medium	Water Absorption result	28 days difference than normal concrete (%)
1	[8]	Bacillus Sphaericus	10^6 cells/ml	Trypticase soy broth	Reduce	50
		Bacillus subtilis		Nutrient broth agar	Reduce	50
		Bacillus magaterium		Hicrome bacillus agar	Reduce	46

		Bacillus Pasteuri		NH4-YE medium	Reduce	70
		Bacillus colli		BATS media	Reduce	35
		Bacillus flexus		Hicrome bacillus agar	Reduce	40
		Bacillus cereus		Hicrome bacillus agar	Reduce	50
2	[5]	Bacillus Sphaericus	10ml	Bio-reagent (3 g/l)	Reduce	22
			20ml		Reduce	3
			30ml		Reduce	7
3	[14]	Bacillus Cereus	10^1 cells/ml	1g/L yeast extract	Reduce	11
			10^3 cells/ml	3g/L yeast extract	Reduce	11
			10^5 cells/ml	5g/L yeast extract	Reduce	12
			10^9 cells/ml	9g/L yeast extract	Reduce	13
			10^{11} cells/ml	11g/L yeast extract	Reduce	12
4	[15]	Bacillus subtilis	10^4 cells/ml	5 gm/l Peptone	Reduce	50
			10^5 cells/ml	10 gm/l CaCl	Reduce	57
			10^6 cells/ml	5 gm/l NaCl	Reduce	57
			10^7 cells/ml	3 gm/l yeast 1.5 gm/l beef	Reduce	50
5	[16]	Bacillus subtilis	10^7 cell/ml	nutritional Lab's broth 1g Beef extract, 2gYeast extract, 5g Peptone, and 5g Sodium chloride	Reduce	60
6	[17]	Bacillus subtilis + 10% calcined clay	10^8 CFU	0.5gm peptone, 0.5gm sodium chloride, and 0.3gm beef extract	Reduce	10
		Bacillus subtilis + 15% calcined clay			Reduce	15
		Bacillus subtilis + 20% calcined clay			Reduce	20
7	[18]	Bacillus pasteurii	5.5×10^6 CFU/ml	chicken manure effluent (CME) in tap water, 20 g urea, and 25 mM CaCl2	Increase	0

				TSB-urea media (30 g tryptic soy broth, 20 g urea, 25 mM CaCl2)	Increase	6
				NB-urea medium (3 g beef extract, 5 g peptone, 20 g urea, 25 mM CaCl2)	Reduce	0.64
8	[19]	Bacillus subtilis	10^3 cells/ml	beef extract at 1.0 gm/litre, yeast extract at 2.0 gm/litre, NaCl at 5.0 gm/litre, peptone at 5.0 gm/litre, and distilled water	Reduce	16
			10^5 cells/ml		Reduce	27
			10^7 cells/ml		Reduce	36
9	[20]	Bacillus Specie	2–3% of the concentration	sodium alginate beads	Increase	1.28
		Bacillus Pasturii	2–3% of the concentration		Increase	1.3
		Bacillus Anthracis	2–3% of the concentration		Increase	1.29
		Bacillus Subtilis	2–3% of the concentration		Increase	1.34

Figure 1 and 2 is the graph of comparison that shows type of bacteria with water absorption percentage result and water absorption comparison in type of growth medium.

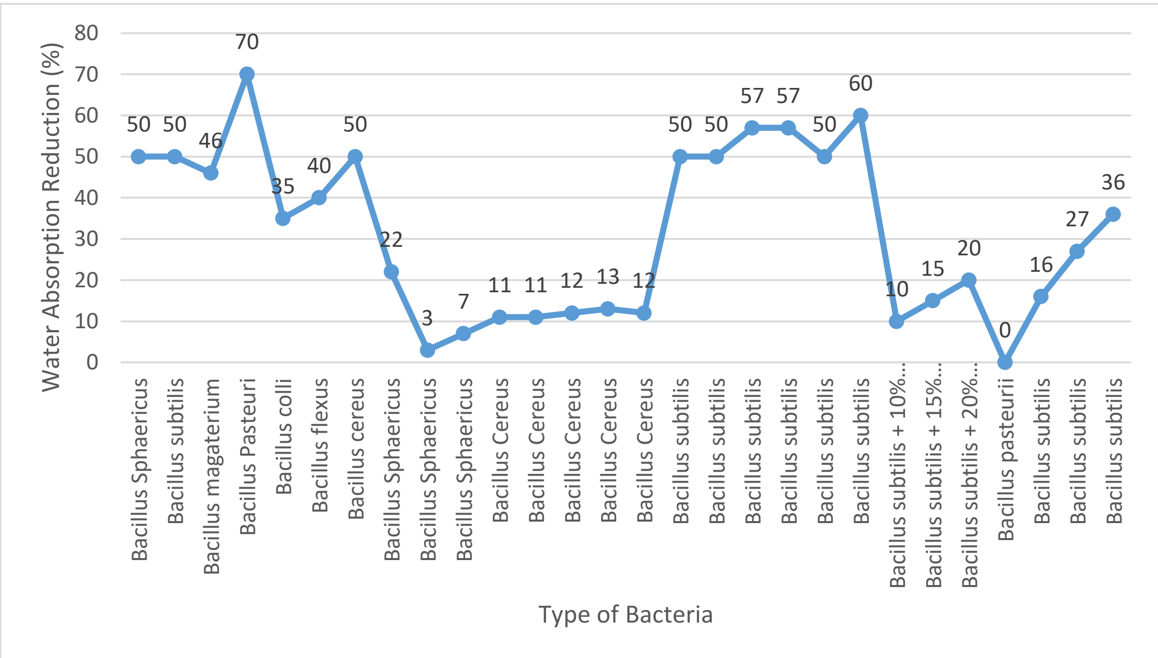

Figure 1: Type of bacteria vs water absorption reduction (%) result in 28days curing

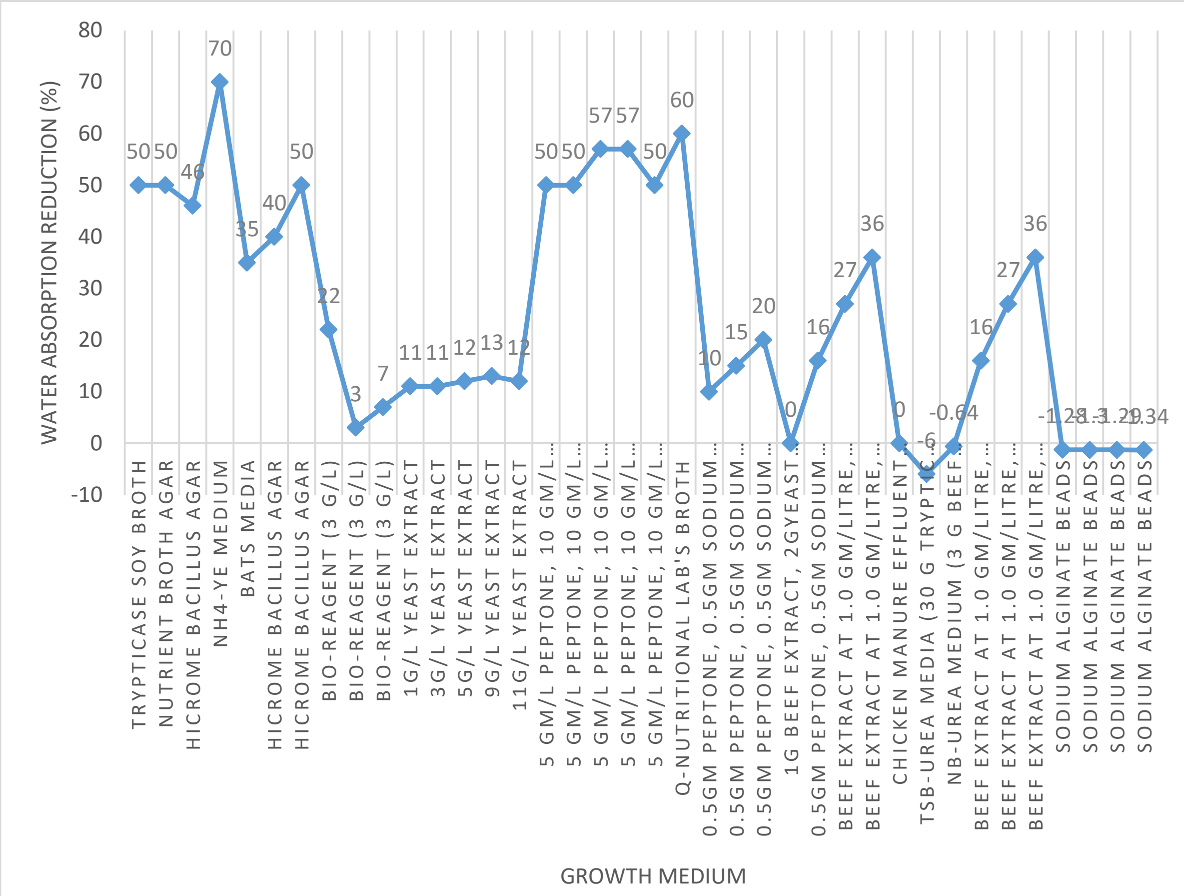

Figure 2: Water Absorption reduction (%) in 28 days curing vs growth medium

4.5 Discussion

After 28 days, it can be seen that the impact of Bacillus strain as self-healing agent has the positive impact to the concrete. According to figure 1 and 2, Abhishek et al. [8] able to find highest water absorption reduction of 70% with selection of Bacillus Pasteuri, cell concentration 10^6 cells/ml, and growth nutrient is NH4-YE medium. While the water absorption increase by 6% , experiment conducted by S. Yoosathaporn et al. [18] with the same species of Bacillus pasteurii and concentration 5.5×10^6 CFU/ml cured with TSB-urea media (30 g tryptic soy broth, 20 g urea, 25 mM CaCl2). Other result resulting in water absorption reduction with range of lowest value 0% and the highest value is 60%.

Apart from that, the water absorption of concrete using Bacillus bacteria is increase. Through experiment conducted by S. Yoosathaporn et al. [18] with species of Bacillus pasteurii and concentration 5.5× CFU/ml and the growth medium is NB-urea medium (3 g beef extract, 5 g peptone, 20 g urea, 25 mM CaCl2). All 4 experiment by Saman Shahid et al. [20] using Bacillus Specie Bacillus Pasturii, Bacillus Anthracis and Bacillus Subtilis with 2–3% of the concentration and nutrient provided is sodium alginate beads increase by value of 1.28%, 1.3%, 1.29% and 1.34% respectively.

In my opinion, water absorption might increase maybe because if the growth medium is not suitable for the bacteria to survive. This may cause void inside the concrete due to growth media added inside the concrete and create passage for water to go inside the concrete. Other than that, the right amount of bacteria and growth medium concentration also important factor that might give the consequences on why the bacteria concrete's water absorption is increase. This is maybe because if there is too much bacteria insert into the concrete mixture, the bacteria will grow more than needed to heal the concrete cracks and cause void inside the concrete to allow water passage seeping through the concrete. As a result, Bacillus strain manage to survive concrete’s high alkalinity environment. Most of reviewed article shows the reduction of water absorption. Finally, from the result, concentration of bacteria, growth medium and and type of bacillus species could be important factor on self-repairing on concrete.

5. Conclusion

As listed in the table and figure above, the type of Bacillus bacteria does not give much impact on the work-ability of bacteria act as self-healing concrete agent in term of durability of concrete through water absorption. But the type of concentration and growth medium is the most crucial part in order to achieve a good result in implementing Bacillus bacteria in concrete. Through all the case study reviewed, concrete embedded with bacteria has a lower water absorption capacity than nominal concrete. This will prevent the concrete from cracking as the porosity of concrete is lower than the normal concrete.

It is proven that Microbiologically Induced Calcium Carbonate Precipitation (MICCP) is long-lasting and effective process of self-healing bacteria concrete. When a crack forms in concrete, the bacteria are activated by air infiltration or moisture, promoting bacterial culture growth inside the concrete matrix. Bacteria's primary job in concrete is to precipitate calcium carbonate. There are many Bacillus bacteria strains, but we only need those that produce carbonate and can survive in harsh environments. When a cracks occurs in concrete, the strains should have a faster growth rate and increased urease activity. To ensure the Bacillus to survive and give positive effect to the durability of concrete is the concentration and the growth medium of Bacillus strain. Most bacteria concentration can survive the alkaline conditions of concrete is 10^1 cells/ml to 10^{11} cells/ml. And most common growth medium that is effective for Bacillus to survive is 20g of yeast extract.

Bacillus species such as Bacillus Subtilis, Bacillus colli, Bacillus Cereus, Bacillus Pasteurii, Bacillus flexus, Bacillus Sphaericus and etc may self-heal cracks in a concrete mass. According to previous research, some bacteria are harmful to human health. Still, others, such as Bacillus Sphaericus, Bacillus pasteurii, Bacillus subtilis, and Bacillus flexus, have no adverse effect on human health and also exhibit a higher capacity for calcite precipitation, making them ideal bacteria for the design of bacterial concrete. Because these Bacillus strains are non-pathogenic and have no adverse effects on humans, they are considered environmentally friendly.

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