RESEARCH ARTICLE

Water Purifying Bio-concrete

Rajiv Gupta*

Department of Civil Engineering, Birla Institute of Technology & Science, Pilani Campus, Pilani 333031, Rajasthan, India

*Corresponding author: E-mail: rajiv@pilani.bits-pilani.ac.in

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Water is the quintessential element of life. In the arid zones like Rajasthan, India, rainfall is scanty and ground water table is too low to satisfy the needs of living beings. One of the water sources is harvesting rain. Though the rainwater does not have its own contaminants but when it touches the surface, it gets contaminated (physically, chemically and biologically). The water thus harvested is used for drinking/ domestic purposes. Drinking water need to be freed from microbial/ disease. Literature indicates the usage of bacteria for self-healing concrete but no study indicates the use of bacteria, *Bacillus Licheniformis (BL) or Bacillus Subtilis* (BS), for the purification of water. These bacteria can survive high temperatures and can exist in spore form to resist harsh environments or in a vegetative state when conditions are good. In present study two bacteria BL and BS are tested. These bacteria feed on the organic contaminants present in water and produce harmless end products. Cultured bacteria are mixed in concrete to make slabs or sprayed on plain slabs with different combinations. The efficiency is analysed by testing the polluted water samples before and after passing through the slab. Results indicate that water is potable.

Introduction

According to UN SDG report, an estimated 663 million people were using unimproved water sources or surface water in 2015. Water-borne diseases still cause huge death to worldwide, making water purification a pressing exploration space. World Bank estimates that 21% of communicable disease in India are related to unsafe water. In India 1600 deaths per day are accounted due to diarrhea. Generally, water in village is accessible either through wells or from surface water. Surface water is exposed to several contaminants. As per Central Pollution Control Board 2011, 80% of India's surface water is polluted. It is estimated that nearly 135 million lives will perish if no action is taken (Gleick 2002).

These facts demonstrate the demand for provision of clean potable water to a huge population that is far-flung with it.

Biological treatment, introduced as trickling filter has been used in Europe to filter surface water for drinking purposes since the early 1900s. A bio filter is a bed of media on which microorganisms attach and grow to form a biological layer called biofilm. These microorganism accumulate to form poly-microbial aggregate such as film, floc, mats or sludge (Flemming and Wingender 2010)

It was found that in most biofilms the composition of microorganism is nearly 10% whereas matrix accounts for approximately 90% (Flemming and Wingender 2010). Bio filtration is thus usually referred to as a fixed–film process. This bio-film comprises of different community of microorganism (bacteria, fungi, yeast etc.) apart from several macro-organism such as worms, protozoa and extracellular polymeric substances (EPS) (Flemming and Wingender 2010). Microbial activities affects the efficacy of the bio filter that highly depend upon microbial activity and a constant source of substrates (Chaudhary 2003). Bio filtration is thus usually referred to as a fixed–film process.

From the purification study of pervious concrete, (Obla 2007) pervious concrete is considered to be the effective mean for green and sustainable growth as an experimental test (Ju Lee *et al.* 2013) was done on pervious concrete by passing a sulfuric acid with pH 2 that enhances its pH value to over 6.5 Pervious concrete is highly porous having void content varying from 18% to 35%.

Conventional biological filter units are intended for large scale and involves hefty capital investment. It is also problematic to sustain a good balance between required flow rate and purification level of output water in such filters.

Invention proposed uses affordable and effortlessly accessible material, which will help beneficiaries to avail this technology. Porous concrete has been progressively used as pavement material to capture surface runoff. It can act as a fixed bed for many microorganisms, which will ultimately digest organic matter and purify the water

To overcome the problem of maintaining optimal flow rate certain bacillus based microorganism can be mixed along with concrete that is effective in decomposing organic matter and a significant reduction in certain disease causing bacteria was obtained. The invention proposed use of certain bacillus based microorganisms that can be mixed along with pervious concrete. It has been found that birds dropping is considered to be one of the chief contaminant of surface water. Avian salmonellosis has been renowned in starlings (Feare 1984). This disease is found to be transmissible in humans, poultry and livestock. When

bacillus tested on broilers there was significant reduction of nearly 60% in Salmonella-positive drag swabs on comparing it with control bird having 100% presence of Salmonella (Knap *et al.* 2011).

Bacillus subtilis exhibit more than one pH optimum for growth depending on the growth conditions, particularly nutrients, metal ions, and temperature. Therefore, the term "alkaliphile" is used for microorganisms that grow optimally or very well at pH values above 9. The pH of concrete varies from 12-13. Bacteria of the Bacillus species have exactly the right characteristics. They can grow in alkaline environments. Their spores can survive for decades in a kind of sleep mode, without food or oxygen. In concrete, they will only come to life if water and oxygen are 'added' in the environment. Bacillus Licheniformis known for its degradation action is cultured. It can sustain high alkaline conditions and high temperature making its growth feasible in concrete.

Methodology

It relates to system for water purification by using bacillus bacteria. Bacillus bacteria is cultured in concrete. In order to grow bacteria in concrete, suitable bacteria (Bacillus Licheniformis and Bacillus subtilis) was cultured in lab and the suspension of bacteria was either mixed with concrete or sprayed on concrete. Raw water contains several microorganism and organic matter that acts as substrate for these cultured bacteria. This substrate come in contact with cultured bacteria, thereby making these bacteria to again come to life. As discussed, due to the degradation behaviour of Bacillus Licheniformis, the microorganism and the organic matter present in raw water are degraded by the cultured bacteria forming a bio film over the time. This bio film (Schmutzedecke) consists of biologically active material that again aids in breaking down of the organic matter.

The method adopted by Indian Standards – Concrete mix proportioning guidelines (**IS-10262:2009)** has been embraced to design the pervious filter slabs. Pervious concrete is made in a similar manner as normal concrete with variation in quantity of fine sand or fly-ash content. Pozzolana Portland cement (PPC) was used along with various cementitious material such as silica fumes, fly ash, and blast furnace slag. Fine aggregate is negligible in pervious concrete and coarse aggregate is kept to narrow gradation. Aggregates passing 10mm and retained over 6mm were used as coarse aggregates. In between the two concrete slab curtains fine aggregate was added maintaining coefficient of uniformity below 3.0. The effective size of grain was kept ranging from 0.13 to 0.62 mm for significant coliform removal efficacy.

The system consist of an assembly comprises of two chambers in a tank as shown in **Fig. 1**. The partition between the chambers is separated by porous concrete slab/s. In between the concrete slab fine aggregate of designed coefficient of uniformity is filled. This partition allows the water to flow into second chamber due to hydraulic gradient formed or through gravitational force when water is filled in the first chamber of the tank. The bacterial suspension is either mixed in porous concrete or it is sprayed on the porous wall. Water is filled in the input chamber and allowed to pass through the porous wall into output chamber.

Several embodiments were proposed with different design composition of pervious slab filter and results were compared for each. In first embodiment two layers of porous concrete separators were provided which consist of input and output portion. The gap between the two separators is filled with fine aggregate. Raw water is filled in input section and the output portion is for receiving filtered raw water. Here the separator A is a concrete mix with bacterial spore whereas separator B is concrete with a designed pore size. In another embodiment, both the separators are formed by concrete mix with bacterial spore. In yet another embodiment separator A is concrete mix with bacterial spore and whereas interior portion of the separator B is sprayed with suspended bacterial pallets solution.

In another embodiment filters were placed horizontally and water was allowed to pass through it. With different size of filter and their composition results were compared for both bacillus subtilis and bacillus licheniformis.

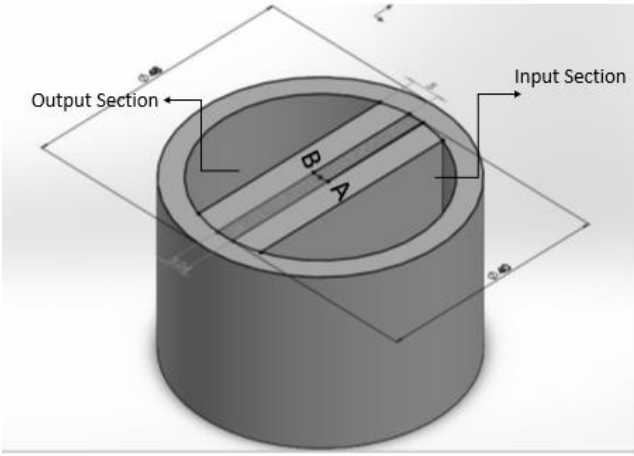

Fig. 1. Test Tank Design (Isometric View).

Results and discussion

When placed horizontally the flow rate obtained was greater than when placed vertically. It is confirmed by changing the configuration and indicated in **Table 1** (Col. Related to BS: E_1, and E_{1A}). The result obtained shows that there was a significant increase in pH value of water from 7 (Inlet value) to average value increase of 9.5 (Outlet value). **Fig. 2** indicates the pH values of treated water. The pH of output comes down to average 8.4 after 2-3 cycles of filtration. The reason being that concrete pH value lies near 12 to 13, making it highly alkaline medium. The solution for this could be addition of certain acidic medium which adds hydrogen ion to water. Sodium sulphate can be added to while mixing it along with certain super plasticizers.

Initially seven combinations were prepared (one control with no bacteria on any slab; six combinations: slab A is concrete mixed bacteria and slab B is plain concrete: 3 for BS and 3 for BL) and initial testing, four best combinations are selected (2 for BS and 2 for BL) along with three controls. One is with plain concrete slab, second with bacteria mixed concrete in vertical position, and third one in horizontal position.

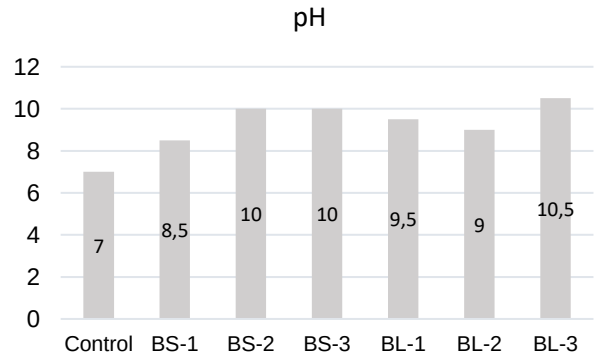

Fig. 2. pH value.

There was significant reduction on 5-day Biological oxygen demand (BOD) in water showing a rise in dissolved oxygen. The 5-day BOD value drops from 0.3 ppm to 0.1 ppm as shown in **Fig. 3**.

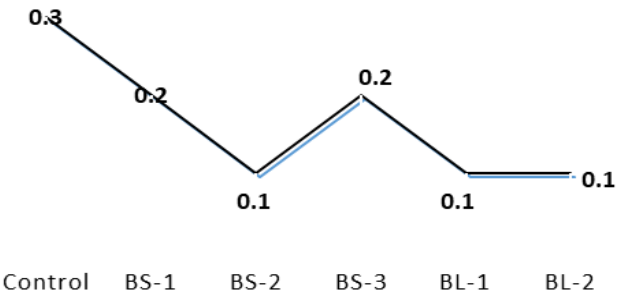

Fig. 3. Biochemical Oxygen Demand.

The E. coli concentration was significantly reduced to a value of zero colonies per 100 mL of water. A 24 hour (**Fig. 4**) and 48-hour (**Fig. 5**) coliform count results in zero E coli colonies depicting significant reduction in microbial content.

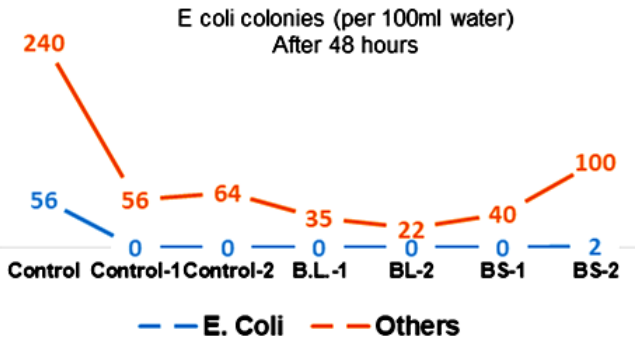

Fig. 4. E Coli (After 24 hours).

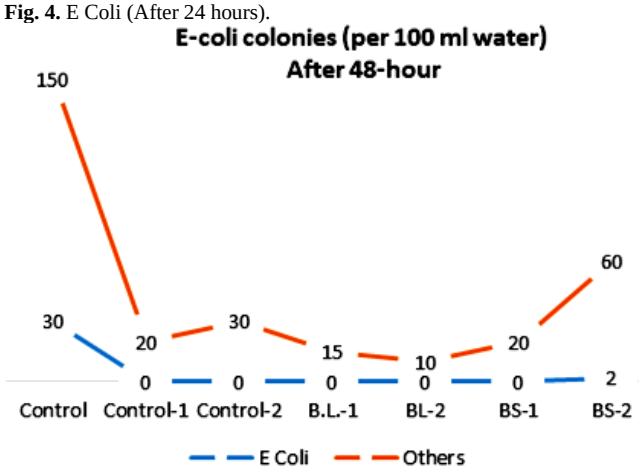

Fig. 5. E Coli (After 48 Hours).

Table 1. Test Results.

Type of Bacteria			Bacillus Subtilis				Bacillus Licheniformis		
TANK No. and (Type of Embodiment)			1 (E_1)	2 (E_{1A})	3 (E_2)	4 (E_3)	5 (E_1)	6 (E_2)	7 (E_3)
S.No.	Facets	Base	NA				NA		
1	Composition Slab A (cultured bacterial solution/ cement)	NA	1:4.4 (0.327)	1:4.4 (0.327)	1:0.5: 3.5 (0.263)	1:5.5 (0.40)	1:4.4 (0.31)	1:0.5: 3.5 (0.263)	1:5.5 (0.4)
	Slab B (water/ cement)	NA	1:0.6:4 (0.308)	1:0.6:4 (0.308)	1:0.6:4 (0.308)	1:0.6:4 (0.37)	1:0.6:4 (0.308)	1:0.6:4 (0.308)	1:0.6:4 (0.37)
2	Water/ Cement	NA	0.327	0.327	0.263	0.40	0.31	0.263	0.40
3	Flow Rate (L/min)	NA	0.21	0.3	0.04	NA	1.5	0.33	0.3
4	pH	7	8.5	10	10	NA	9.5	9	10.5
5	Hardness(mg /L as $CaCO_3$)	148	84	80	88	NA	100	144	96
6	Chloride Content	64.9	110	116	99.96	NA	124	89.97	123
7	BOD	0.3	0.3	0.1	0.2	NA	0.1	0.1	0.3
8	E Coli concentration After 48 hrs. (colonies/ 100mL)	3	Nil	Nil	N.C.	NA	1	35	3
9	Other Bacteria	68	Nil	Nil	N.C.	NA	17	>150	68

Inference

As mentioned due to high pH value of concrete there was significant rise in pH value. This limitation can be handled by using 1% to 2% sodium sulphate solution along with water. There was significant reduction in hardness of water though the chloride content was found to be increased in all the tanks. There was substantial decrease in biochemical oxygen demand depicting an increase in dissolved oxygen value. There was negligible fluoride content in rainwater. The small increase in its value in all tanks can be accounted to slabs that had attached fluoride salt crystals. Nitrate was found to be below detectable limit chemically concluding absence of any microbial activity in water. E. coli has expressively achieved to zero coliform colonies per 100 ml water sample. Also other microorganisms have been significantly reduced. All the test results are shown in

Table 1. After initial tests (**Table 1**), four combinations were selected: (i) E1 and E2 with BS and (ii) E1 and E3 with BL. They are designated as B.S. - 1 and - 2 and B.L. -1 and -2. After conducting number of tests, average results of E-coli are indicated in **Table 2** and **Table 3** after 24 hours and 48 hours respectively. Here, one slab (A) is mixed with bacteria and second slab (B) is sprayed inner surface (facing slab A) with bacteria. It can be observed that though both *Bacillus Licheniformis* (BL) or *Bacillus Subtilis* (BS) bacteria are reducing the concentration of E coli. However, in few cases, some minor E coli count is found using BS. Hence, it is recommended to use BL for purifying the water for drinking purpose.

Table 2. E Coli results after 24 hours.

S.No	Sample	Bacteria		Average	
		Coliform(E.coli)	Other		
1	Control	30	150	15	75
2	Control – 1	0	20	0	25
3	Control -2	0	30		
4	B.L -1	0	15	0	12.5
5	B.L -2	0	10		
6	B.S -1	0	20	1	40
7	B.S -2	2	60		

Table 3. E Coli results after 48 Hours.

S.No	Sample	Bacteria		Average	
		Coliform(E.coli)	Other		
1.	Control	56	240	28	120
2.	Control – 1	0	56	0	60
3.	Control -2	0	64		
4.	B.L -1	0	35	0	13.5
5.	B.L -2	0	22		
6.	B.S -1	0	40	1	70
7.	B.S -2	2	100		

E Coli After 48 Hours

Conclusions

Harvested rainwater may get contaminated with biological components, which may cause fatal diseases. To use it for drinking, microbial/ disease causing contaminants should be completely removed. An inbuilt purification system is developed in water tank by the action of *Bacillus Licheniformis* (BL) or *Bacillus Subtilis* (BS) either mixing with concrete or spraying on the surface for cleaning the water. It is observed that BL survive in the prevailing conditions and results are positive. Values are within the permissible limits, and show that after filtration, water is potable. Field tests are conducted and it is observed that the system is working efficiently. It can be concluded that BL-2 should be used to clean the water (BL-2 is two slabs system, with slab A is mixed with BL and slab B's inner surface is sprayed with BL)

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Appendix

1. Design of Pervious Concrete slab

1.1 The method adopted by Indian Standards – concrete mix proportioning guidelines (**IS-10262:2009)** has been finally embraced to design the filter slabs

1.2 The mean target strength ft from the specified characteristic compressive strength at 28-day fck.

$$F_t = f_{ck} + 1.65\ S$$

1.3 Take significant w/c ratio

1.4 **Calculation of water content:** Approximate water content for 10mm max. Size of aggregate = 208 kg /m^3

1.5 **Calculation of cement content:**

$$\frac{\text{Water content per m3 of concrete}}{\frac{Water}{cement}}$$

1.6 Finally, coarse aggregate is computed

2. Culture of Bacteria

Culture of Bacteria: In order to culture the bacteria in concrete, the suitable bacteria (Bacillus Licheniformis) is cultured in the lab (**Fig. 6**) and the suspension of bacteria is sprayed on concrete. Detailed procedure is given below:

2.1 Autoclave the whole apparatus required and oven dry them

2.2 For 20 ml of the solution add 0.5 grams of LB Broth powder in the conical flask.

2.3 In case the culture is to be grown in Petri dishes, 2% Agar is added to the solution.

2.4 Autoclave the solution to kill any remnant microorganisms. This process also dissolves Broth.

2.5 After autoclaving, take the whole apparatus to the UV Chamber. The UV Chamber need to be switched on before 15 min of the process so that bacteria in that vicinity are cleared.

2.6 Transfer the Broth solution to test tubes and inoculate the bacterial sample using micro pipette. Carefully discard the micro pipette tips after use.

2.7 In case of growth in Petri dishes, liquid agar needs to be poured in the plates and once its gets solidified, streaking process of bacteria is undertaken.

2.8 Keep the test tubes in the vibrator at 37.7 ^{0}C (Optimum growth temperature of bacteria).

2.9 Overnight culture is obtained and inoculated again for the secondary growth in 250 ml Broth solution.

2.10 After two- three hours when the optical density of the culture is in such a way that bacteria is growing in Logarithmic phase, take out the culture from vibrator. In Log phase bacterial number doubles, since this the active growth phases, bacteria in this phase are chosen to culture on concrete.

2.11 Clean the culture off media. In order to do the same, suspend the medium in PBS (Phosphate Buffered Saline) and centrifuge the suspension.

2.12 Remove the medium from the tube and suspend the culture in PBS again and repeat the process of centrifugation.

2.13 Finally clean the culture with PBS and suspend the bacterial culture free form media in Saline. This suspension is transferred to a sterile sprayer and to be sprayed on Concrete.

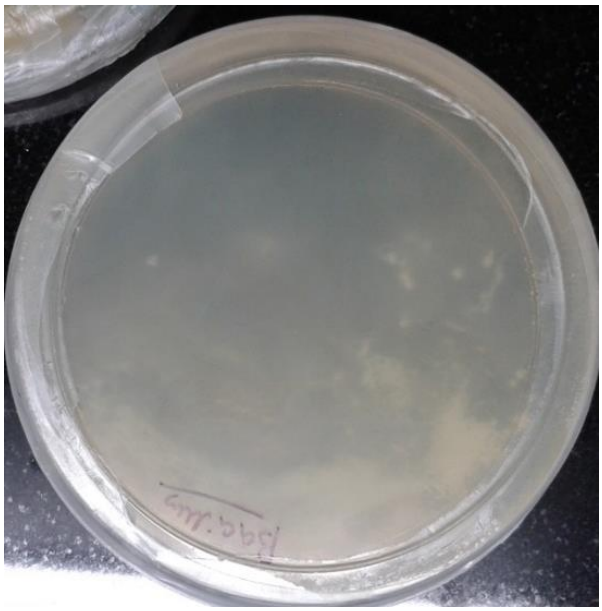

Fig. 6. Bacillus Licheniformis.

Keywords

Water purification, bio concrete, pervious concrete, bacillus bacteria.

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References

1. Chaudhary, D.S.; Vigneswaran, S.; Ngo, H-H.; Shim W.G.; Moon, H.; *Korean J. Chem. Eng.*, **2003**, *20*, 1054.
2. Flemming, H.; Wingender, J.; "The biofilm matrix." Nature reviews. Microbiology, Nature Publishing Group, **2010**, 8, 623.
3. Gleick, P. H.; Dirty Water : Estimated Deaths from Water-Related Diseases 2000-2020. Pacific Institute Research Report, **2002**
4. Indian concrete mix design guide lines, **2009**.
5. Ju Lee, M.; Gin Lee, M.; Huang, Y.; Liang Chiang, C.; *International Journal of Engineering and Technology*, **2013**, *5*, 532.
6. Kna, I.; Kehlet, A. B.; Bennedsen, M.; Mathis, G. F.; Hofacre, C. L.; Lumpkins, B. S.; Jensen, M. M.; Raun, M.; Lay, A.; *Poultry Science*, **2011**, *90*, 1690.
7. Obla, K. H.; "Pervious Concrete for Sustainable Development." Recent Advances in Concrete Technology, **2007**, 151.

Authors Biography

Rajiv Gupta (Senior Professor of Civil Engineering, BITS, Pilani) completed his B.E., M.E and Ph.D. from BITS, Pilani. In his last 35 years of teaching and research, he has published more than 150 research papers, guided more than 10 Ph.D. scholars, taught more than 30 courses, and reviewed more than 150 books, project, and papers of reputed journals. He is involved in number of research and development projects worth more than Rs. 1000 lacs of World Bank, UGC, DST, University of Virginia, and other sponsored organizations. He has implemented a multi sustainable Rainwater Scheme in 10 villages. He worked as Dean of Engineering Services and Hardware and was instrumental in developing Goa, and Hyderabad campuses, apart from other different construction at Pilani campus.

Graphical abstract

Water is the quintessential element of life. In the arid zones like Rajasthan, India, rainfall is scanty and ground water table is too low to satisfy the needs of living beings. One of the water source is harvesting rain. Though the rainwater does not have its own contaminants but when it touches the surface, it gets contaminated (physically, chemically and biologically). The water thus harvested is used for drinking/ domestic purposes. Drinking water need to be freed from microbial/ disease. Literature indicates the usage of bacteria for self-healing concrete but no study indicates the use of *Bacillus Licheniformis (BL) or Bacillus Subtilis* (BS) bacteria or for the purification of water. These bacteria can survive high temperatures and can exist in spore form to resist harsh environments or in a vegetative state when conditions are good. In present study two bacteria BL and BS are tested. These bacteria feed on the organic contaminants present in water and produce harmless end products. Cultured bacteria are mixed in concrete to make slabs or sprayed on plain slabs with different combinations. The efficiency is analysed by testing the polluted water samples before and after passing through the slab. Results indicate that water is potable.