Bioluminescent Bacteria in a Closed Environment an Approach for a Biolight Source

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Abstract—Energy problem is the trap of modern sociality where light costs much, in efficient and causes pollution. In this approach closed Eco-spheres were utilized to support life for *Vibrio azasii* a light-emitting bacterium using simple, recycled and cheep supplies. The light produced by the system is not bright yet usable in terms of lighting the streets, ornamental and night signs. Considering its low production price, long life span expectancy, Eco-friendly and it is sustainable only by day light as an energy source. During this study new opportunities arose regarding the use of *V. azasii* bacterium as a biodetector for heavy metal pollution in water and soil and production of omega-three essential fatty acid by means of fermentation. Finally the use of the closed ecosystems itself as a "Noah's ark" representative for the microbiome flora of its sampling zone for post-catastrophe bio-remediation.

Key words — Biolight, *Vibrio azasii*, closed ecosystem, biodetector, street lighting.

1 INTRODUCTION

The bacterium *Vibrio azasii* is a Gram-negative, rod-shaped that bioluminesces through a population-dependent mechanism called quorum sensing. Colonies of *V. azasii* are collectively luminescence upon reaching a certain cell density (Stevens & Greenberg, 1997 and Hastings & Greenberg, 1971).

The bacterial luminescence reaction, catalyzed by luciferase, involves the oxidation of a long-chain aliphatic aldehyde and a reduced flavin mononucleotide (FMNH2), generating luciferin (FMN), the oxidized form of the aldehyde, and water, with the liberation of excess free energy in the form of a blue-green light at about 490 nm (Eberhard *et al.,* 1981) FMNH2 + RCHO + O2 ----> FMN + RCOOH + H2O + light (490nm)

The bioluminescence intensity reflects the overall health of the organisms and the luminescence reaction, which reflects metabolism, is sensitive to a wide variety of toxic substances (Stevens& Greenberg, 1997 and Eberhard *et al*., 1981). This sensitivity has made them a popular choice for methods to detect environmental pollutants, such as heavy metals and pesticides.

Nelson *et al.* (2003) reported that small closed ecological chambers are an efficient model for developing principles of bioregenerative systems. Knowing these principles can enhance long distance space travel and remotehuman habitation. Closed systems were constructed in the laboratory using tissue culture flasks and one-liter glass bottles. All nutrients, micro algae and aquatic macro-invertebrates were added approximately one week before sealing. Viability of the closed system was judged by continued survival and activity of macro-invertebrates.

The same authors studied varying light intensity, nutrient concentration, temperature and varying air space. They found that *Tigriopus californicus* (a salt water copepod detritus feeder) populations maintained a steady abundance at high intensity light, increased quickly at moderate light and declined quickly at low light. Daphnia magna (a fresh water zooplankton) population increased more rapidly at high temperature. Within the levels of nutrient concentration they tested*, T. californicus* population growth increased with more algal nutrients. While trying to test the relationship between air space and viability, they observed that when refuges were present for algae and macro-invertebrates, populations outlived those without refuge space.

On other hand, Globus (1970) individuate that Orbital space settlements will be located between the planets. While the Sun will provide ample reliable energy, there are essentially no material resources in the immediate vicinity. All materials will need to be transported from Earth, the Moon, the asteroids, comets, or other planets and their moons. Thus, the space colony designer may assume ample energy but must conserve materials. Therefore, the life support system of the colony should recycle all materials. Since a life support system consisting primarily of plants, animals, and single-celled organisms, life support system may be described as an ecosystem. Because the space colony's ecosystem is preferred does not import or export materials, it's called a closed ecosystem.

Globus added that the Space settlement ecosystem components may comprise as the following:

1. A biotic - Nonliving Components a Light a)

Atmosphere b) Soil chemistry c) Water d) electromechanical devices

2. Biotic - Living Components a) Plants b)Animals c) Microorganisms.

The author also described the closed Eco-system construction and how the development of its components could be observed For example the closed eco-system could be prepared as follows:

1.Get a large clear bottle with a cap, 2. Collect several plants, small animals, some soil, and some water. The water may contain pond scum or other living material, 3. Place the collected materials into the bottle, 4. If possible, puts a thermometer and/or other instruments into the bottle so that you can read them after the bottle is sealed, 5. Put the cap on the bottle and seal the space between the cap and bottle with melted wax or other air-tight material, 6. Place the bottle where it will receive at least indirect light. This is the energy source. While development of such eco-system is observed throw the following steps, at regular intervals record the ecosystem's condition, note the temperature and instrument readings if available, describe the color and other observable characteristics of the ecosystem's contents, Enter this information into a notebook, If quantitative information is recorded, create a chart of the data and calculate the mean, standard deviation, and range, Several other studies were also preformed to evaluate the end results of similar closed eco-systems pointing out that Egland and Greenberg (2001); Kempner and Hanson (1968) and Brown (2010) were nearly exact methods were repeated to develop ecosystems in this study.

On the other aspect, regarding bacterial bioluminesces Lin and Meighen (2009) reported that there are three major genera, into which most luminous bacteria are classified, *i.e. Photobacterium*, *Vibrio*, and *Photorhabdus*. Species existing in the marine environment are mainly categorized into the *Photobacterium* and *Vibrio* genera, and the terrestrial species are classified into the *Photorhabdus* (previously designated as *Xenorhabdus*) genus. Species within the *Photobacterium* genus are generally light organ symbiosis of marine animals, whereas the *Vibrio* species exist as free-living forms as well as symbiosis in the *V. fischeri* is a bioluminescent bacterium that participates in a communication phenomenon known as quorum sensing.

Luminous bacteria reside in symbiosis on a pair of light organs in the mantle body of the squid. Utilization of the illumination function is believed to frighten nearby predators, allowing the squid to escape. Complementary to this West (2006) regarding quorum sensing and light-emission in *V. fischeri* as (Fig. 1).

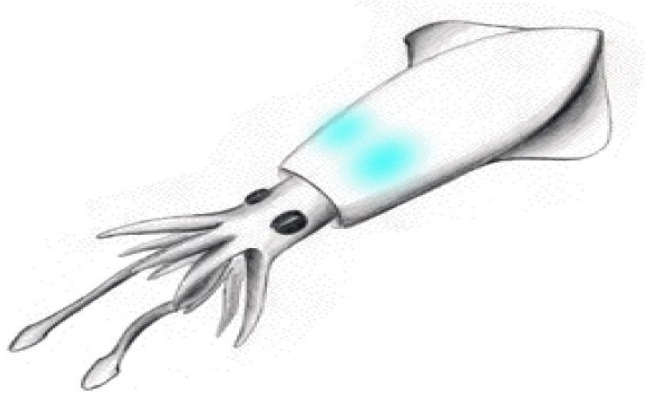

Fig. 1. Big fin squid.

The variation in the light output of these organisms individually will give insight into biological gene noise. For a study of noise to succeed, *V. fischeri* must be cultured in conditions that induce maximal intensity and duration of light output. The purpose of this project was to identify all pertinent environmental factors and characterize their effects on the luminescence of *V. fischeri*. Further insight on the isolation, classification and use of *V. fischeri* in heavy metal detection and omega 3 production was mentioned (Bartlett, 1999).

2 MATERIALS AND METHODS

A fast quantitative diabetic strip test was used to determine glucose, ketone, acetone, pH, and protein in solution named “combi test strips” and these were used to deduce the situation of closed ecosystems.

2.1. Proteinase K Protocol:

The method was described by Li *et al.* (2016). Proteinase K Protocol, produced by the fungus *Tritirachium album* Limber, is a serine protease that exhibits very broad cleavage specificity. It cleaves peptide bonds adjacent to the carboxylic group of aliphatic and aromatic amino acids and is useful for general digestion of protein in biological samples. It has been purified of RNase and DNase activities. The stability of Proteinase K in urea and SDS and its ability to digest native proteins make it useful for a variety of applications, including preparation of chromosomal DNA for pulsed field gel electrophoresis, protein fingerprinting and removal of nucleases from preparations of DNA and RNA. A typical working concentration for Proteinase K is 50–100µg/ml.

2.2. Automated Sanger sequencing:

Sanger sequencing is a method of DNA sequencing, based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication. Developed by Sanger *et al*., (1977) , it was the most widely-used sequencing method for approximately 25 years. More recently, Sanger sequencing has been supplanted by "Next-Gen" sequencing methods, especially for large-scale, automated genome analyses. However, the Sanger method remains in wide use, primarily for smaller-scale projects and for obtaining especially long contiguous DNA sequence reads (>500 nucleotides).

2.3. The used medium:

The isolation medium used in this study was obtained from Atlas (1989) as showed in Table (1).

Table 1:The components of the used medium.

Component	Quantity
Aquarium sea water salt	33 g
Tris-hydroxymethyl aminomethane	6 g
Ammonium-chloride	5 g
Tryptone	5 g
Yeast extract	5 g
Glycerol	3 g
Calcium-Carbonate	1 g
Agar-Ager powder	20 g
Manage pH around	7.2

3 RESULTS AND DISCUSSION

Each couple of this tables and graphs is a representative about a sample named after its place (zone from where it was took) showing its progression throw out days until arrested stationary phase is reached “real closed sustainable Eco-sphere” or otherwise death “Eco-spher failure”

Table 2: Chemical analysis of Alexandria water sample.

Date	Glucose (mg/dL)	Protein (mg/dL)	pH	Ketone (mg/dL)	(density) total	Suger (mg/dL)
05/03/12	<50	5	7.5	5	1027	<50
07/26/12	<50	5	7.5	5	1027	<50
07/28/12	<50	30	7.5	5	1027	<50
07/31/12	<50	30	7.5	5	1027	<50
08/05/12	<50	30	7.5	5	1027	<50
08/16/12	<50	30	7.5	5	1027	<50
08/30/12	<50	30	7.5	5	1027	<50
09/05/12	<50	30	7.5	5	1027	<50
09/12/12	<50	30	7.5	5	1027	<50
09/19/12	<50	100	7.5	5	1027	<50
09/27/12	<50	100	7.5	5	1027	<50

In the previous tables the chemical analysis of key components of Eco-spheres were analyzed and recorded to give a picture about the overall health of it.

Were pH lowering, total sugar sacristy, ketone and protein increase where all sines of a failing eco-sphere.

Table 3: Chemical analysis of Matroh water sample:

Date	Glucose (mg/dL)	Protein (mg/dL)	pH	Ketone (mg/dL)	(density) total	Sugar (mg/dL)
05/12/12	<50	5	7	-	1026	<50
07/26/12	<50	5	7	5	1026	<50
07/28/12	<50	5	7.5	5	1026	<50
07/31/12	<50	30	7.5	5	1026	<50
08/05/12	<50	30	7.5	5	1026	<50
08/16/12	<50	30	7.5	5	1026	<50
08/30/12	<50	30	7.5	5	1026	<50
09/05/12	<50	30	7.5	5	1026	<50
09/12/12	<50	30	7.5	5	1026	<50
09/19/12	<50	30	7.5	5	1026	<50
09/27/12	<50	100	7.5	5	1026	<50

Table 4: Chemical analysis of Hardaga water sample.

Date	Glucose (mg/dL)	Protein (mg/dL)	pH	Ketone (mg/dL)	(density) total	Suger (mg/dL)
07/20/12	-	100	8	7	1029	-
07/26/12	-	100	7.5	7	1029	-
07/28/12	-	300	7	7	1029	-
07/31/12	-	300	5	7	1029	-

*only these tables were included in the paper as representatives thaw more than 10 ecosystems were sampled.

For example Hardaga water sample form day one had a very bad profile it had undetectable sugars and high ketone content, even thaw a ceramic ring and sea-shell crush were intrudes to the system to control the pH (as all other systems) yet the buffering effect failed to push off the fast dieing eco-sphere.

The mixes were made in the sake of more divers eco-spheres were the diversity is a ground breaking advantage for any eco-sphere to compete against harsh out door on street environment as each and every organism give an upper hand by means of its unique genotypes compared to the others enabling brake down of complicated material and building up of new ones On the other hand two mixes failed Alexandria-Matroh mix and Alexandria-Fayed mix that could be a result of in compatibility of organisms were they ecologically were lethal to one another or the eco-sphere's material itself were toxic to new organisms.

Isolation of the *vibrio*:

Living Squids were brought from Port-said put on ice and transferred to the laboratory where Isolation media was prepared in Petri-dishes where this protocol as shown in (Fig. 2.) where used to isolate the bacteria.

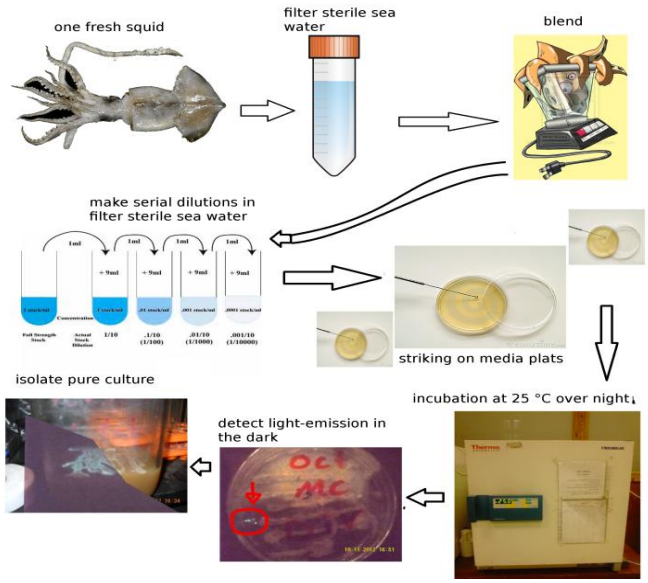

Fig. 2. Isolation protocole.

Introduction of the bacteria to the closed ecosystem:

when the vibrio were introduced to the systems it had to be gradually so as not to shock the system out of balance liquid cultures were centrifuged out on low speed (to avoid cell damage) and the pelt was injected into the systems by a syringe (1 ml for each bottle) ,then they were left to stabilize for a week if the system did not glow another shot were given to the system till vibrio had numerical advantage to keep up with system's other components and flora as shown in (Fig.3).

Fig. 3. The eco-spheres glowing after the vibrio where introduced.

Identification:

Firstly the bacteria were simple stained and gram-stained and microscopically examined to certify its morphological features. Simple stain (A) foxin red, (B) gram-stain of the bacterial cultures as shown in (Fig. 4).

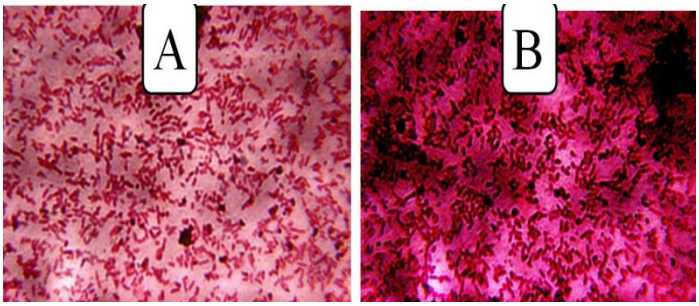

Fig. 4. Simple stain A (foxin red), B gram-stain of the bacterial cultures.

Then RNA was isolated, rivers translated and PCRed by a universal 16S-rRNA primer the PCR product was checked by gel that it is stable (one band) as shown in (Fig.5) then was send to “Applied Biotechnology Trading co.”(Address: Egypt , Ismailia , 87 El-Nour and El-Ghaba street, Elsalam) for sequencing as shown in (Fig.6) was deposited in the GenBank at The DNA Data Bank by accession no.: (KF018175).

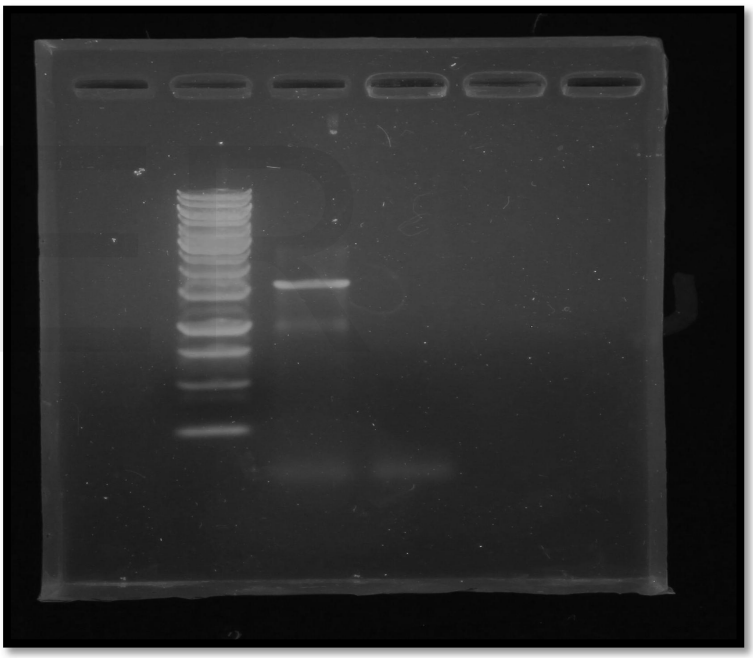

Fig. 5. band on gel, 50-ladder marker on left.

Forward
"NNNNNNGGGCGCAGCTANCATGCAAGTCGAGCGG
AACGAGTTATCTGAACCTTCGGGGAACGATAACGGC
GTCGAGCGGCGGACGGGTGAGTAATGCCTAGGAAAT
TGCCCTGATGTGGGGGATAACCATTGGAAACGATGGC
TAATACCGCATAATGCCTACGGGCCAAAGAGGGGGA
CCTTCGGGCCTCTCGCGTCAGGATATGCCTAGGTGGG
ATTAGCTAGTTGGTGAGGTAAGGGCTCACCAAGGCG
ACGATCCCTAGCTGGTCTGAGAGGATGATCAGCCACA
CTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCT
GATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGG
GTTGTAAAGCACTTTCAGTCGTGAGGAAGGTAGTGTA
GTTAATAGCTGCATTATTTGACGTTAGCGACAGAAGA
AGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATA
CGGAGGGTGCGAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCATGCAGGTGGTTTGTTAAGTCAGATGTGAA
AGCCCGGGGCTCAACCTCGGAATTTGCATTTGAAACT
GGCAGANTAGAGTACTGTANAGGGGGGTAGAATTTC
AGGTGTAGCGGTGAAATGNGTAGAGANCTGANGGAN
TACCNNTNGCNAAGGNGGCCCCCNT"

Reverse
"NNNNNNNNTNNANANANNTTGNTTAAGCGTCCTC
CCGAAGGTTAAACTACCTACTTCTTTTGCAGCCCACT
CCCATGGTGTGANGGGGGGTGTGTACAAGGCCCGGG
AACGTATTCACCGTGGCATTCTGATCCACGATTACTA
GCGATTCCGACTTCACGGAGTCGAGTTGCAGACTCCG
ATCCGGACTACGACGCACTTTTTGGGATTCGCTCACT
CTCGCAAGTTGGCTGCCCTCTGTATGCGCCATTGTAG
CACGTGTGTAGCCCTACTCGTAAGGGCCATGATGACT
TGACGTCGTCCCCACCTTCCTCCGGTTTATCACCGGCA
GTCTCCCTGGAGTTCCCGACATTACTCGCTGGCAAAC
AAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCA
ACATTTCACAACACGAGCTGACGACAGCCATGCAGC
ACCTGTCTCAGAGTTCCCGAAGGCACCAATCCATCTC
TGGAAAGTTCTCTGGTTGTCAAGAGTAGGTAAGGCTC
TTCGCGTTGCATCTAATTATACCACATGCTCCACCGTT
TGTGCGGCCCCCCGTCAATTCATTTGAGTTTTTTTTT
CTACCGCACTTTCNGGTGTTCTACATAATCGTTAGCTC
CGAAAGCCACG"

Fig.6. The 16S band glowing under UV in geldoc sequencing result.

This sequences were compared with BLAST® on NCBI data base giving this two possible results as shown in (Figs.7& 8).

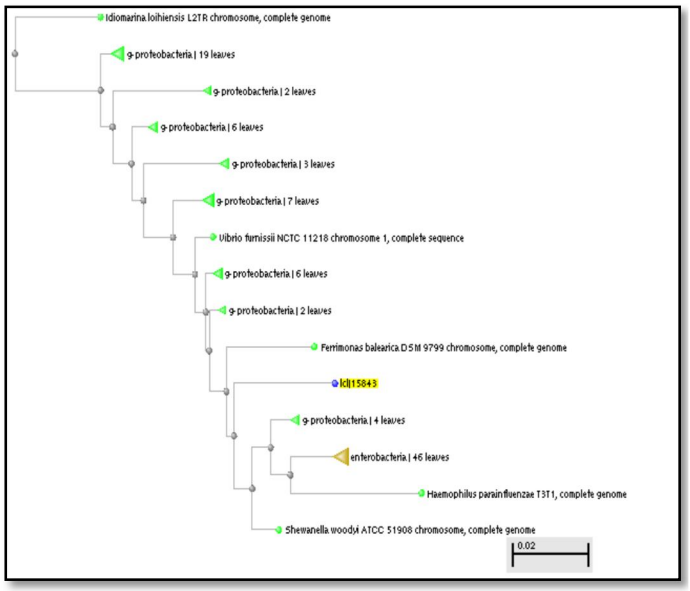

Fig. 7. NCBI gave the sequence the ID# lcl|15843 where it reveals having common ancestor with *Vibrio harveyi* ATCC BAA-1116 [g-proteobacteria] taxid 338187.

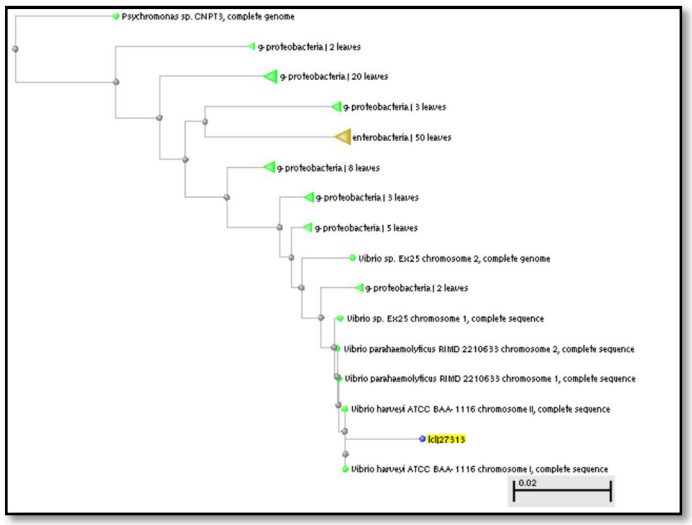

Fig. 8. NCBI gave the sequence ID# lcl|27313 were it reveals having common ancestor with *V. harveyi* ATCC BAA-1116 [g-proteobacteria] taxid 338187.

According to this results the Isolated bacterium is unknown to NCBI in terms of it Bing more than 2% different from any other organism on the data base.

Linking between *V. harveyi* and *V. fischeri* Sequence was submitted to gene bank and got accretion No. (KF018175).

Tests for other applications and technologies :

Application 1

as a food additive the light-emission mechanism of the bacterium is as follows (fig.9).

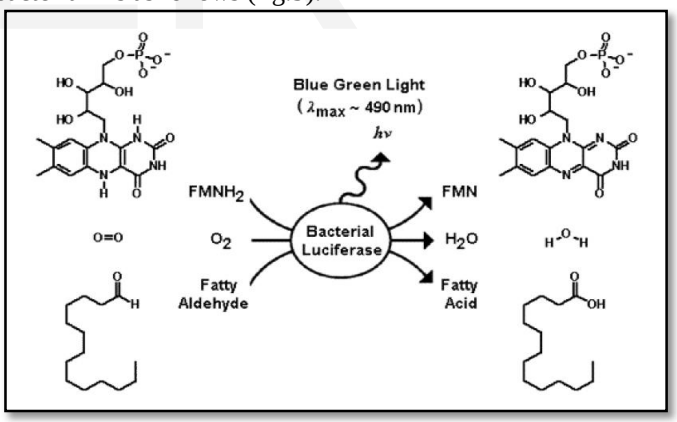

Fig. 9. The light emitting reaction is the same reaction that produce unsaturated fatty acid from fatty aldehyde.

In the shadow of this information bacterial broth cultures were intubated in shaking incubator for 3 days, centrifuged out of solution, then sonic-disruption were applied to cell biomass.

After the sonic bath the cells were centrifuged again on 1500RPM for 10 min at 4°C to separate all the fatty acids out the top layer were sucked out by a syringe and a drop was injected in a TLC paper against two controls (Palm oil as saturated control , fish oil ω−3 control)left to run over night as shown in (Fig.10).

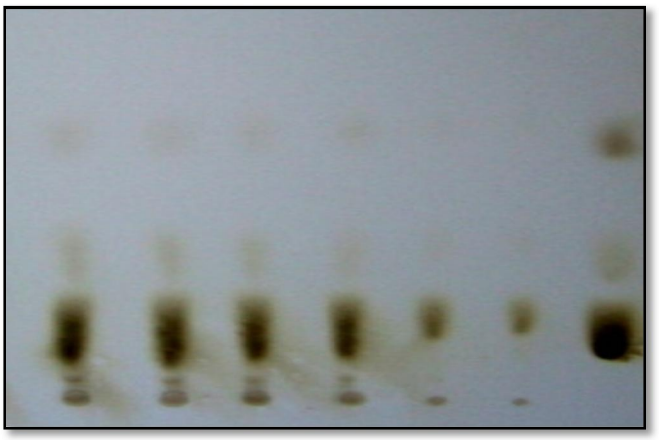

Fig. 10. From right to left palm oil , fish oil (band 1),fish oil (band 2) then 4 replicas of the bacterial oil.

note: bacteria is not tested for Algiers, till this is confirmed only oil extract should be added to food.

Application 2

Serving as a contamination fast detection kit Both *Vibrio harveyi* and *vibrio fischeri* are known to be extremely sensitive to toxins and heavy metals in there environment were there light-emission reflect there over all health on this info this protocol was developed as follows (Fig.11).

- Incubate cultures over night to get a glowing slant culture.
- Get water samples that are to be tested.
- If the water samples are fresh water add 3% NaCl to sample (3g to 100ml).
- Pour samples on the slants and shake.
- Observe light.

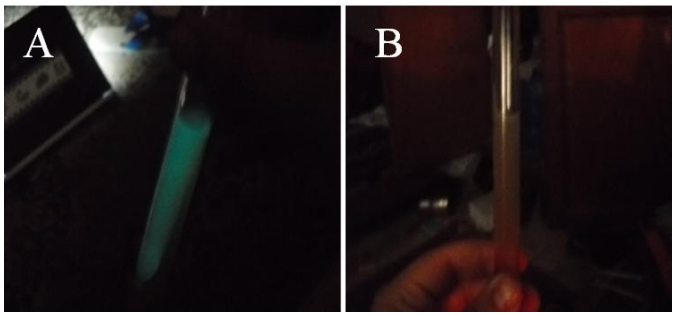

Fig. 11. A glowing, B not glowing; if it remains glowing its chemically safe, only minor filtration is needed to make it drinkable but if the light die out that indicate the unpalatability of water were it is contaminated by toxin or heavy metal other tests need to be done on any water that causes the light to go out to make proper treatment protocol thus providing a fast cheep on demand test that could be applied anywhere with low costing tech.

4 RECOMMENDATIONS

The closed ecosystems are easy to make and have a grate potential of serving as a “Noah's Ark” for its sampling zone where it carries almost all essential Micro-flora representatives for this zone with no maintenance cost at all, this is helpful in terms of post-catastrophe bioremediation and re habitation of native organisms for fast environmental healing Vibrio are easy to ferment on a relatively cheap marine biowasts and can give valuable essential oils that could be laterally used as food additives and supplements Vibrio natural sensitivity for contaminants make it fast pollutants director that need almost no lab gear Threw out all this project bio-light seemed like a usable light source it just need its chance and much more research.

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