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Minute Microbial Levels Detection in Water Samples by Portable Microbe Enrichment Unit Technology

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Abstract

Water purity requires continuous monitoring. PMEU technology (Portable Microbe Enrichment Unit) provides the means for starting the enrichment and analysis immediately after the sampling. The entire procedure can be automated and microbe levels monitored in real-time from the enrichment broth by using IR, optical, UV or volatiles screening. For example, single cells of *Escherichia coli* have been verified from water samples in less than 10 hours in an official validation project. Also such pathogens as *Salmonella*, *Campylobacter* and *Yersinia* have been detected rapidly from natural, purified and irrigation waters. The PMEU technology has potential in the continuous monitoring within water departments and distribution systems, as well as in environmental studies.

Keywords: rapid microbe detection, water hygiene, indicator bacteria, ColilertTM, PMEU, environmental monitoring, pathogens, water distribution

1. Background

In the present manuscript experimental approach and work carried out with the Portable Microbe Enrichment Unit (PMEU) is presented. This device is an ideal tool to investigate micro-organisms life at microscopic and molecular scale. Micro-organisms such as bacteria have a large influence on our lives with impact on economy, various industries, healthcare, agriculture and forestry, as well as in water management. In Finland our team published booklet entitled “Vesien Mikrobiaapinen” (in English: “A Microbiological ABC book of the waters”) with the purpose to provide microbiological information to decision makers. As scientists we continuously deal with the public and their representatives: the political decision makers. In order to get funding, we ought to make them understand the importance of environmental protection and conservation of water sources, from the microbiological point of view.

One area where micro-organisms have a large influence is the threat and deleterious impact on human and animal health caused by pathogenic micro-organisms. They are potentially distributed via waterways and often disseminated at small concentrations which still pose health hazards. In order to detect these minute concentrations, new tools are needed, also to simulate their biochemical activities. Their detection is not either a single or a simple event but a whole diagnostic process (Figure 1). Focusing on the often neglected step of specimen collection is of crucial importance. Sampling should be carried out considering all the physicochemical parameters, selection of sampling site etc. Usually it is required to transport and store the sample with caution and care. This is the step when most of the microbes present in the sample are often destroyed. They simply cannot survive or do not remain microbiologically viable. In order to avoid these problems, the Portable Microbe Enrichment Unit was developed. Time is saved in the PMEU processing of the samples, since the verification steps could follow sooner compared to traditional microbiology. The basic simple idea is to start the enrichment cultivation right subsequent to sampling. In practice, using the PMEU technology accelerates the growth and metabolism of microbes in a way that as a whole has been designated as “enhanced enrichment”. It has also opened up new applications in the field of water microbiology, which are briefly introduced in the present article.

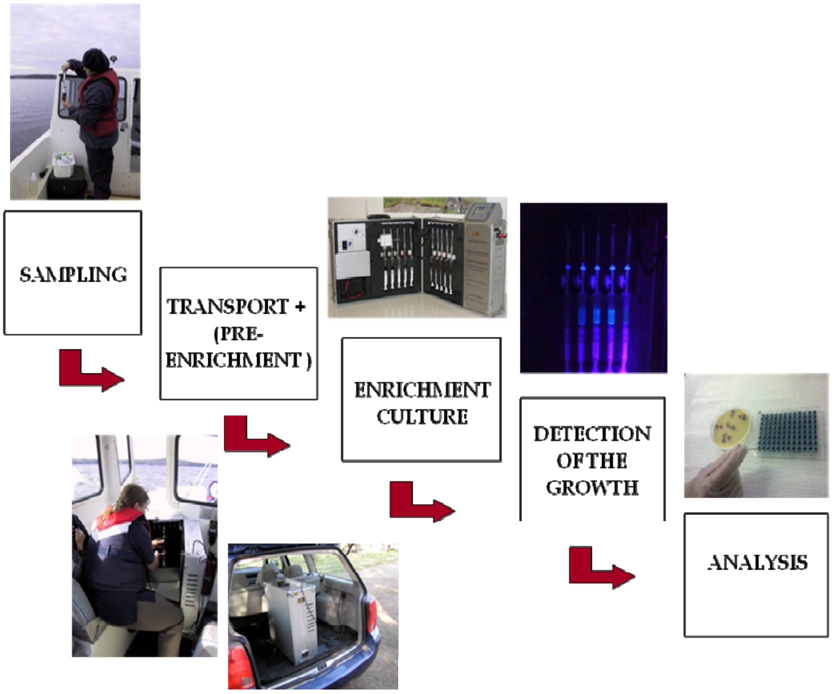

Figure 1. Microbial detection process by the PMEU

2. Basics of the PMEU Technology

The ideas of bringing the cultivation step close to the sampling site, and to carry out it with enhanced enrichment technology has been developed in the laboratory of Finnoflag Oy. According to PMEU technology, samples are collected into specific syringes that are further positioned into the described apparatus for cultivation according to the selected temperature program (Figure 2). In diverse PMEU equipment, microbial growth and biochemical passageways are monitored by optical sensors, including infrared and ultraviolet sensors, and also by means of volatile compounds (see Figure 3). In most cases the detection time is shortened at least by 50%, as the microbial growth is enhanced by the specific conditions in the PMEU. Very often the detection time is shortened from weeks to days and from days to hours. If a non-selective medium is used, the enhanced growth of various sample microbes can occur simultaneously.

Figure 2. Sample syringes inside the PMEU Spectrion® (Samplion Oy, Kuopio and Siilinjärvi, Finland)

Figure 3. Various PMEU units: PMEU Scentrion® (on the left), PMEU Spectrion® (middle) and PMEU Basic version

PMEU technologies consist of different strategies in monitoring the enhanced microbial growth and metabolism (Hakalehto, 2010). The essential point is the option to boost the microbes at cellular level. Various standard media can be used for cultivations in an effective way. One example is the application of Colilert^{TM} medium which is being used for the monitoring of coliforms and *Escherichia coli* simultaneously (Eckner, 1998). These bacteria belong to the universal hygienic indicators group. Growing coliforms produce yellow color when incubated in Colilert^{TM} medium and simultaneously UV fluorescence is produced by *Escherichia coli* if present (Figure 4). These reactions can be measured by the PMEU Spectrion® and the PMEU Coli-line automated version (Figure 5). PMEU Coli-line can also be equipped with Automated Sample Collection System (ASCS), a system also pertinent for other PMEU versions, both for water and gaseous samples. In practice, the submerged broth culture allows more cells of the family *Enterobacteriaceae* to be recovered than in the plating methods (Pesola, Vaarala, Heitto, & Hakalehto, 2009). The direct plating could produce direct estimates of the microbial concentrations, but they have a poor recovery of the viable but not culturable (VBNC) microorganisms (Rompré, Servais, Bandort, de-Roubin, & Laurent, 2002). In the PMEU method the original cell densities could be extrapolated according to the growth curves of the cultures (Hakalehto, 2011a).

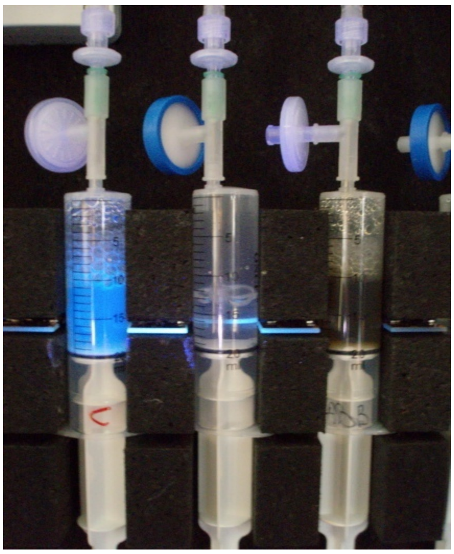

Figure 4. *E. coli* detection with Colilert^{TM} broth in PMEU Spectrion®. The positive fluorescent culture can be seen on the left syringe as a result of bacterial enzymatic activity (Berg & Fiksdal, 1988). This fluorescence is produced by the *E. coli* ability to cleave the substrate (MUG) resulting in the formation of the fluorescent product 4-methylumbelliferone (American Public Health Association, 1995)

Figure 5. PMEU Coli-line version based on the PMEU Spectrion® technology equipped with ASCS (Automated Sample Collection System). This experimental arrangement was tested during the year 2011 in a pilot of a water plant at Savonia Technical University in Kuopio, Finland. This device had automatically monitored water quality at the plant, and circulated information about any instability or reduced microbiological quality before the water is pumped into the network and to consumers

A whole spectrum of different bacteria can be studied by the PMEU apparatus. Besides bacteria, yeasts, molds, and other kinds of eukaryotic microbes can also be monitored. In principle, there is a question of understanding how dynamic the microbial metabolism is, anabolism, catabolism and overflow metabolism all being studied with various PMEU versions. Investigation of these metabolic pathways opens up a view on the mechanisms of pathogenesis and symbiotic cooperation between these microbes and their hosts. In order to investigate human, animal, plant, or microbial viruses in the PMEU it is needed to establish cell cultures of appropriate hosts for their propagation.

Recently, the PMEU Spectrion® method was validated by the State Research Centre of Finland (VTT) (Wirtanen & Salo, 2010) (Figure 6). Their results indicate that observable growth starting from individual *E. coli* cell was obtained in 10 hours. In addition, monitoring of hospital hygiene using both the innovative sample collection practices with the PMEU syringes and fast cultivation of samples in the PMEU Spectrion® units have been successfully tested (Hakalehto, 2006; Hakalehto, 2010; Pesola et al., 2011). The membrane filter techniques produce detectable colonies within a longer period of time (Hsu & Williams, 1982). Different microbial species could be enumerated at the same time, even though there is a risk of overgrowth when the membranes are moved for cultivation onto the Petri dishes. On the other hand, it is possible to use the short enrichment period of few hours in the PMEU for simultaneous propagation of all strains on a generalized bacteriological medium (Hakalehto, 2010). The exact cell numbers during the PMEU cultivation can be obtained by inoculation of the enrichment broth onto the plates (Wirtanen & Salo, 2010). In this case, the pre-enrichment in the PMEU has been documented to improve the recovery of the bacterial cells 2.5 fold in comparison with the direct plating (Pesola et al., 2009).

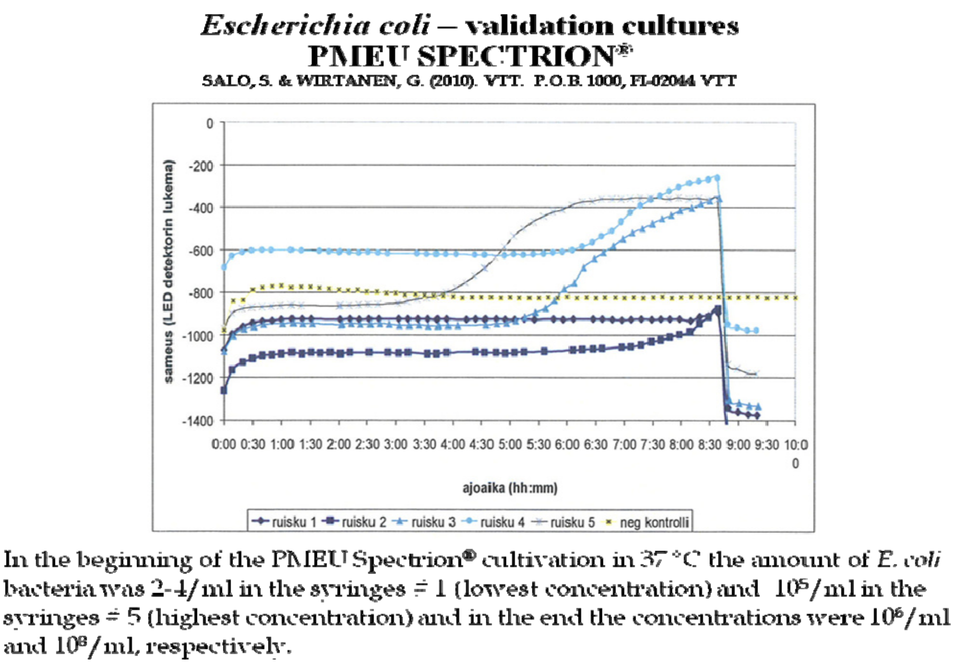

Figure 6. PMEU Spectrion® validation experiment with *E. coli* (Wirtanen & Salo, 2010.)

All concentrations of this indicator bacteria were detected within 9 hours from the experiment onset.

The most sensitive version PMEU equipment is the PMEU Scentrion® which has been used to screen for neonatal septicemia (Hakalehto et al., 2009; Pesola et al., 2011), as well as to study the intestinal microflora (Pesola et al., 2009; Pesola & Hakalehto, 2011; Hakalehto & Hänninen, 2012) (Figure 7).

Volatiles from the PMEU culture were studied by gas sensor technology (ChemPro100i® Chemical Detector, Environics Oy, Mikkeli, Finland) (integrated in a PMEU Scentrion® prototype, Samplion Oy, Siilinjärvi, Finland). This detector system was equipped with a delay line to maintain the relative humidity constant during the measurement and to record evaporating substances. The gas flow into the sensors was directed through the valves from different enrichment syringes. Measurements were based on the relative increase of the resistance on the probes caused by the presence of the volatiles. These alterations were detected with three MOS (Metal Oxide Sensors) and two ScC (Semiconductive Cell) sensors.

Bacterial metabolism is always accompanied by volatiles production. Different bacteria produce different volatiles and combined as mixed cultures they may produce different volatiles compared to pure state (Hakalehto, 2010; Hakalehto et al., 2010; Hakalehto, Jääskeläinen, Humppi, & Heitto, 2012). Therefore, it is fascinating to simulate different real life conditions in the PMEU environment, and to study how different bacteria do behave together in nature or in our body (e.g. in gastrointestinal system). This approach is also providing a deeper insight into the relations between the members of the human normal flora, and how it contributes to prevention of infections.

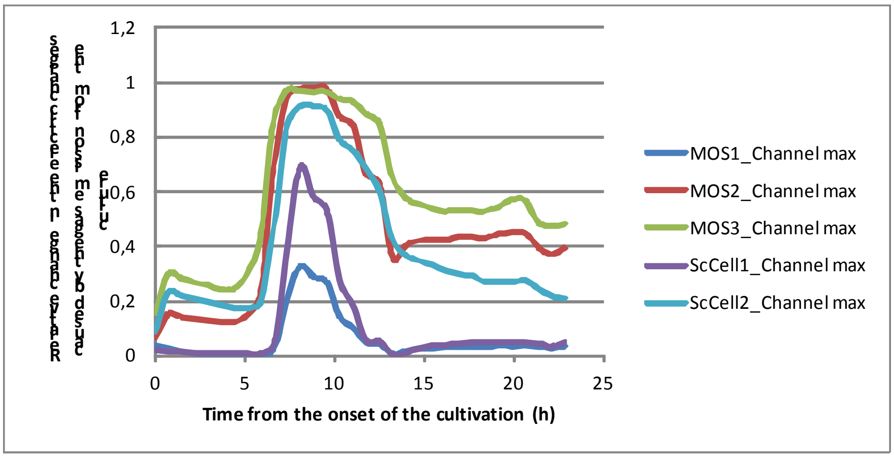

Figure 7. Detection of the gaseous emissions from a mixed culture of *Clostridium butyricum* and *Lactobacillus brevis* in the PMEU Scentrion®

This experiment showed a dramatic onset of growth of an anaerobic organism in about 6-7 hours. In this case the lactobacilli provoke the onset of clostridial growth (Hakalehto & Hänninen, 2012). This boosting effect, however, prevents the latter from sporulation, and from causing permanent problems by rooting into the gut. The effect is based on the production of carbon dioxide by the LAB (lactic acid bacteria). Different lactobacilli have been recently found to reside also in the gastric areas of healthy individuals (Hakalehto, Vilpponen-Salmela, Kinnunen & von Wright, 2011b).

3. PMEU in Water Microbiology

Water management requires awareness for hygienic emergencies. Recently, in Finland a wide national project focusing on this problem (called the Polaris study) has been carried out. The PMEU Spectrion® and the automated PMEU Coli-line version have been involved in the project in several water departments. The PMEU enrichment results have been screened wirelessly (via the internet) in real-time by all participants (Figure 8). It enables the establishment of an early-warning system with stand-alone PMEU devices in the water management. The remote controlled PMEU can be automatically connected to other controlled equipment that may add the purification chemicals (disinfectants) if required by the presence of bacterial or other microbial contamination.

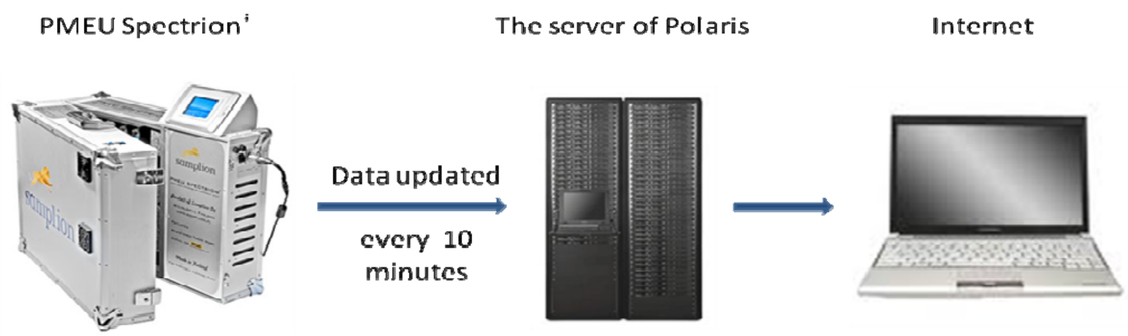

Figure 8. The results of cultivation are reported on-line in the Internet

This specific PMEU approach had been provisionally tested in a water distribution network system in Kuopio City, Finland (with 100.000 inhabitants and 350 km of pipelines) (Hakalehto et al., 2011c). Both hygienic indicators and various pathogens, such as Salmonellas, Campylobacteria, Yersinias, Staphylococci, and *Bacillus cereus* have been effectively monitored (Hakalehto, Pesola, Heitto, Närvänen, & Heitto, 2007; Hakalehto et al., 2009; Pitkänen et al., 2009; Mentu, Keitel, Heitto, & Hakalehto, 2009). For example, a single *Salmonella* cell produced a detectable signal (infrared light or ion mobility sensors or traditional medium plate confirmation) in about 10 hours (Hakalehto et al., 2011c).

The PMEU technology had also been used to monitor water circulation system of paper and pulp industry. PMEU equipped with detection techniques such as ATP monitoring and immunoassays have been implemented for the studies on *Bacillus* sp. and other process industry contaminants (Mentu et al., 2009).

PMEU system has been used for tracing the waste water distribution in case of natural waters contaminations by indicator bacteria (coliforms and enterococci) (Heitto, Heitto, & Hakalehto, 2006). Linked to the above-mentioned approach, it is also possible to track the source combined with phenotyping methods (Heitto, Heitto, & Hakalehto, 2009). In this study it was possible to distinguish between the enterococcal load originating from municipal sources and the one originating from industrial effluents in the lake water surrounding these facilities. Continuous monitoring of bacterial pollutant strains present in water distribution and in waste water systems could be practiced with the PMEU technology (Hakalehto et al., 2011c).

4. PMEU in the Developing World

PMEU has been applied abroad in several projects abroad under harsh field conditions (e.g. in Burkina Faso, Central Africa, where pathogens detection was difficult in an irrigation water flow system). The detected pathogens belonging to the genera *Yersinia* and *Campylobacter* were dispersed via the irrigation water onto vegetables and caused frequent epidemics. In this case, PMEU was used for selectively enrich these pathogens. Most of other bacterial growth in this pool originated from the city waste waters, also concentrated under these tropical conditions. Consequently, the water was directly used for irrigation where more than likely those pathogens were being also distributed in spite of their non-cultivability by traditional means. As a result, National Institute of Health and Welfare of Finland researchers implemented the methods developed in Finland by Finnoflag Oy for their field work with the PMEU Spectrion® in order to acquire evidence on the dissemination of selected pathogens.

5. Simulations of the Alimentary Microbiome

In microbiological studies detection is only the first step before one can proceed to identification and further biochemical and physiological characterization. There is a need to comprehend how bacteria function both outside (environment) and inside of human bodies which is one of the corner stones in environmental health studies (Hakalehto, 2012). Humans are interconnected with environment through their microbes. In our intestines there are trillions of different bacterial cells continuously active. In order to open up a window into their world, we have to understand their contribution to our health. Many intestinal bacteria can be called "opportunistic pathogens". However, they do not cause any visible diseases since they are under the control of the whole present microbial community. The eminent Prof. Joshua Lederberg has introduced the concept "Alimentary Micobiome" where the normal flora is considered as an organ of the host system (Lederberg, 2000). The microbial interactions and molecular communication within the host can be simulated in the PMEU equipment, too (Hakalehto, 2012). It can also accelerate not only the microbial growth but all coupled metabolic events, which in turn can be examined in homogenous cultures. Microbes of the gastrointestinal tract strive for a balance which we had designated as BIB (Bacterial Intestinal Balance) (Hakalehto, 2011a). The tendency of mixed intestinal microflora to build up small scale ecosystems has enormous beneficial effects on the host body system, for example preventing pathogens to colonize gastrointestinal system, or to facilitate nutrients absorption by the host. With the PMEU approach, the effects of pH, bile substances, gases, antibiotics and defensins on various bacterial populations, have been studied (Hakalehto, Humppi, & Paakkanen, 2008; Hakalehto et al., 2010; Hakalehto, 2011a, b). Various isolates from the endoscopic and colonoscopic specimens were also investigated by PMEU methods (Hakalehto et al., 2011b; Hell et al., 2010). Finally, in the healthcare sector and also in the environment, the dissemination of antibiotic resistant strains can be monitored by the PMEU at a much lesser cost (Hakalehto, 2011b).

6. Example on Microbial Influences on a Larger Scale

Although in the microbiology the focus is in the microscopic creatures, they may have vast influences on our lives in global scale which need to be monitored. In order to understand the effects of invisible micro-organisms on our lives, giant viruses (giruses) give a good example (Figure 9). These viruses were reported in the summer of 2011 in the American Scientist (Van Etten, 2011). In this report, a new group of viruses has been recognized, called at present giant viruses or giruses. These organisms have remained unobserved and neglected by scientific world because of their size. Virologists commonly use 0.2 micron filters for water samples in order to remove mainly bacteria, therefore micro-organisms larger than 0.2 microns are not expected to go through. These giant viruses can reach a size of about 1 micron in diameter with a genome larger than those of the smallest bacteria. They have been monitored in the Pacific Ocean using metagenomic studies. These organisms have been shown to represent the second most common type of the DNA in the Pacific, next to the even more numerous bacteriophages´ DNA.

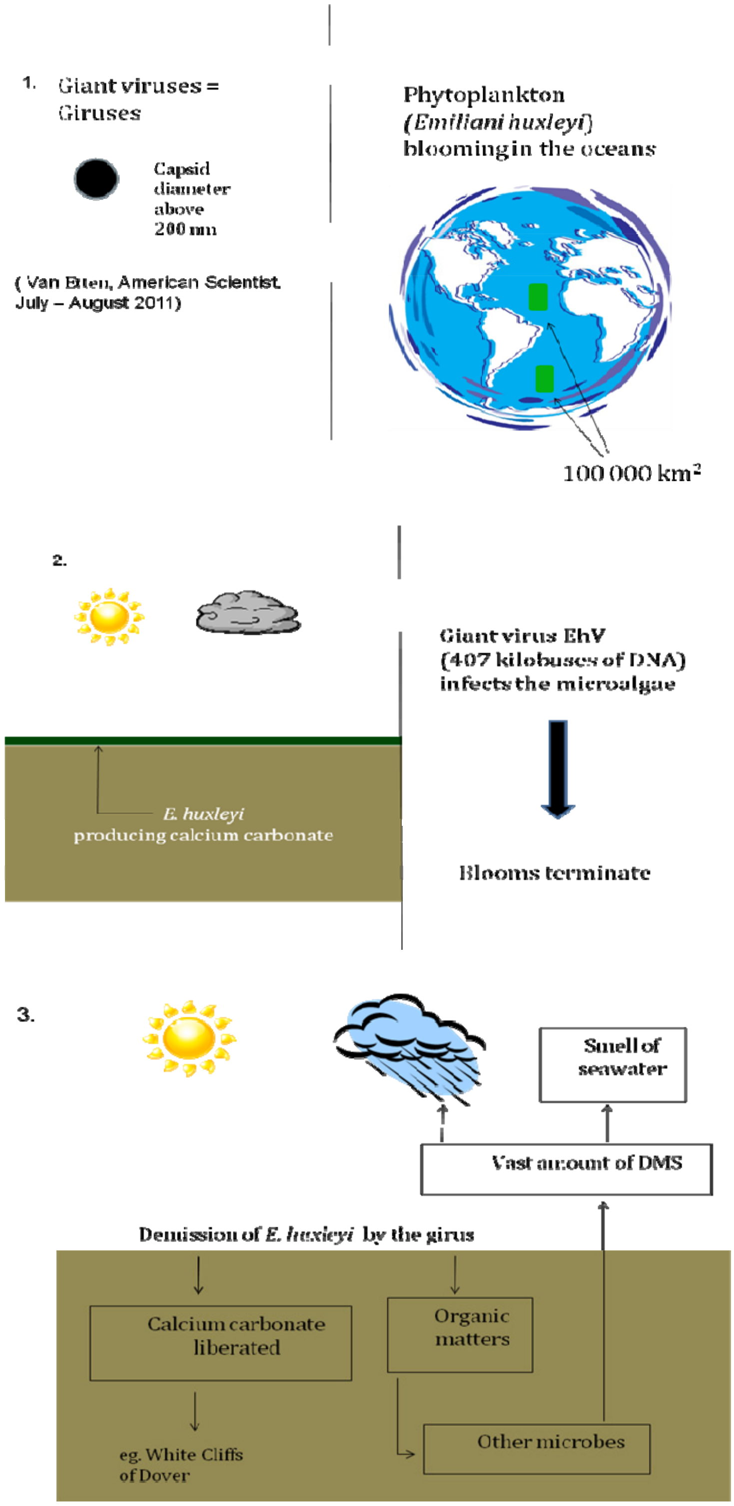

Figure 9. Environmental influences of the giant viruses of phytoplankton (according to Van Etten, 2011)

7. Conclusions

Implementation of PMEU system has been proved to be successful:

1) to automatically collect real-time water samples,
2) in following up the recovery of several strains simultaneously in different microbiological specimens,
3) in taking advantage of the benefits of the submerged cultivation in terms of reliability (2-5 times more strains recovered), speed (enhanced growth and metabolism), and homogeneity,
4) in real-time monitoring of molecular interactions,
5) in field and laboratory versions for verification and source-tracking purposes,
6) in contamination control,
7) in saving lives and costs in hospitals and in environment by screening infections and hygiene, and
8) in screening of the microbiological influences on the ecosystems.

The research on environmental microbiology could provide us with excellent tools for promoting the sustainable development globally.

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