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Technical report on the actual microalgae practices

30th June 2010

Technical report on the actual microalgae practices

Action 1- State of the literature

Action 1 regards the research in scientific literature of pilot and real scale phytodepuration plants operating in zone with cold climate and changeable weather condition, such as Varese Ligure.
Results are summarised as follows:
(1) definition of design parameters (process and installations) emerging from literature;
(2) definition of the climatic and environmental variables affecting the phytodepuration technique with micro algae in the territory of Varese Ligure as required for the implementation of this action.

(1) Definition of design parameters (process and installations) emerging from literature.

PROCESS DESIGN PARAMETERS

From the close examination of national and international scientific literature, it emerges that the most important parameters, that need to be monitored during the phytodepuration processes are temperature, light, nutrients, gas, pH, as described in the following, according to Munez et al. (2006) that listed and described in details parameters that influence the algae process. These information are provided below.

Temperature

The efficiency of microalgae-based treatments normally decreases at low temperatures (Abeliovich, 1986). Muñoz et al. (2004) observed that the removal efficiency doubled when the temperature increases from 25 to 30°C, using a symbiotic microcosm formed by a C. sorokiniana and a R. basilensis strain (the activities of both microorganisms increased with the temperature in the tested range). However, Chevalier et al. (2002) demonstrated that a cold-adapted cyanobacteria strain was suitable for nutrient removal at an average temperature of 15°C. Likewise, Grönlund (2004) described a pilot-scale high-rate algal pond able to permit a 90% BOD removal with 2.5 days of hydraulic retention time at temperature lower than 10 °C and light intensity below 200 µE $m^{-2}s^{-1}$ (Swedish subarctic region, latitude 63°N). These studies therefore showed that with cold-adapted photosynthetic strains in optimized bioreactors for wastewater treatments is possible the decrease in biological activity with temperature inherent to any biological methods.
An excessive temperature at high light intensities and high biomass concentration can also arise from the fact that algae convert a large fraction of the sunlight into heat (Abeliovich, 1986). A temperature control by an external heat exchanger or by water spray have been proposed to ensure a stable microalgal population but costs often remain prohibitive, even for high-quality algal mass cultivation (Tredici, 1999). An alternative to temperature control is the combination of microalgal strains with similar characteristics (in terms of O_2 supply, inhibition and harvesting) but with different optimal growth temperatures (Morita et al., 2001).

Light supply

Sunlight intensity greatly varies during the day and during the year. Algal activity increases with light intensity up to 200–400 µE m^{-2} s^{-1}, when the photosynthetic apparatus becomes saturated, and decreases at higher

light intensities (Ogbonna and Tanaka, 2000b; Sorokin and Krauss, 1958). Photoinhibition has therefore been observed during the central hours of a sunny day when irradiance can reach up to 4000 µE m^{-2} s^{-1} (Rebolloso Fuentes et al., 1999). It is more likely to occur at low microalgal concentration, such as during start-up (Göksan et al., 2003), because the light intensity to which microalgae are actually exposed is not reduced by mutual shading (Evers, 1991; Contreras-Flores et al., 2003; Richmond, 2000). A careful photobioreactor designing can also avoid excessive damage of the photosynthetic apparatus by distributing the light irradiation for a certain land area onto a larger surface (Torzillo et al., 2003). Reducing the size of the antenna of photosynthetic cells using molecular tools reduces light adsorption and usually allows higher photosynthesis rates under high light intensities (Melis et al., 1999).
Periodical absence of light (or periods of low light intensity) cause a halt or a strong reduction of photosynthesis, which generally leads to the occurrence of anaerobic conditions in the reactor. However, photosynthesis and pollutant removal normally resume once light is available again. Waste stabilization ponds are therefore designed to cope with natural diurnal or seasonal light intensity fluctuations by, for instance, increasing the hydraulic retention time (HRT) in the system (Tadesse et al., 2004). High HRT, or the use of storage tanks during period of low light intensities, are also important to avoid increasing in toxic pollutant concentrations and inhibition. In a pilot-scale closed photobioreactor, inoculated with a C. sorokiniana–Comamonas sp. consortium, oxygen production and acetonitrile removal dropped when illumination was stopped for 10 hours, but the process quickly recovered each time illumination was resumed (Muñoz et al., 2005b). Wastewater storage during nigh-time should therefore not affect the overall process efficiency.

pH

Microalgal CO_2 uptake can cause the pH raising to 10–11 in high-rate algal ponds (HRAPs) and high pH values (up to 9) were also recorded during salicylate biodegradation by an algal–bacterial consortium in an enclosed photobioreactor (Muñoz et al., 2003b). This increase, which is beneficial for the disinfection of pathogens, can also cause a decrease in the pollutant removal efficiency (Oswald, 1988; Schumacher et al., 2003) as complete bacterial inhibition at pH above 10 is commonly observed in stabilization ponds (Mara and Pearson, 1986; Oswald, 1988). It is however difficult to dissociate the direct effects of pH on microbial growth from collateral effects such as modifications in the $CO_2/HCO_3^-/CO_3^{-2}$ and NH_3/NH_4^+ equilibriums or in phosphorus and heavy metal availability (Laliberté et al., 1994). The pH also influences nitrogen and phosphorus removal via NH_3 volatilization and orthophosphate precipitation at a high pH (9–11) (Craggs et al., 1996; Garcia et al., 2000b; Nurdogan and Oswald, 1995). Fortunately, it is relatively easy to control the pH in biological systems.

Dissolved oxygen concentration (DOC)

High DOC levels can generate photo-oxidative damage on microalgal cells and therefore can decrease treatment efficiency (Oswald, 1988; Suh and Lee, 2003). For instance, Matsumoto et al. (1996) reported a 98% decrease in the photosynthetic O_2 production rate when the DOC increased from 0 to 29 mg l^{-1} (≈350%). O_2 super-saturation in enclosed photobioreactors designed for mass algal cultivation can reach up to 400%, which severely inhibits microalgal growth (Lee and Lee, 2003; J.S. Lee and J.P. Lee, Review of advances in biological CO_2 mitigation technology, Biotechnol. Bioproc. E 8 (2003), pp. 259–354.Lee and Lee, 2003). Fortunately, O_2 supersaturation does not constitute a severe problem in biodegradation processes due to the continuous O_2 consumption by heterotrophic bacteria. For instance, the DOC was always very low (about 0 mg l^{-1}) during the biodegradation of acetonitrile and salicylate in the batch mode when the pollutants were present and being degraded. However, it also always rapidly increased after complete pollutant depletion (Guieysse et al., 2002; Muñoz et al., 2005). High O_2 concentrations are therefore a good indication of complete pollutant depletion in continuous processes (Muñoz et al., 2004). Further research should be conduced to investigate if the DOC can be used for process control to optimize, for instance, the biomass concentration in the system.

Predators

Infections by parasitic fungi or the development of food chains in the photobioreactor can cause unexpected process failure (Abeliovich and Dikbuck, 1977). Fortunately, these potential problems can easily be avoided by daily operating the process at low O_2 levels for a short period of time (1 hour) in order to suppress the growth of higher aerobic organisms (Abeliovich, 1986).

Microbial interaction

The symbiotic microalgal–bacterial relationship is clear when microalgae provided the O_2 necessary for aerobic bacteria to biodegrade organic pollutants, consuming in turn the CO_2 released from bacterial respiration. However, microalgae and bacteria do not limit their interactions to a simple CO_2/O_2 exchange. Microalgae can have a detrimental effect on bacterial activity by increasing the pH, the dissolved oxygen concentration (DOC) or the temperature of the cultivation broth, or by excreting inhibitory metabolites (Oswald, 2003; Schumacher et al., 2003). They can however enhance bacterial activity by releasing extracellular compounds as shown by Wolfaardt et al. (1994), that observed that diclofop methyl removal by a bacterial consortium increased up to 36% when actively growing algae or their metabolites were added to the culture. Similarly, bacterial growth can enhance microalgal metabolism by releasing growth-promoting factors (Fukami et al., 1997; Gonzalez and Bashan, 2000) or by reducing O_2 concentration in the medium (Mouget et al., 1995; Paerl and Kellar, 1978). De-Bashan et al. (2002), for instance, reported that the presence of Azospirillum brasilense enhanced ammonium and phosphorous removal by C. vulgaris. Bacteria can also inhibit microalgae by producing algicidal extracellular metabolites (Fukami et al., 1997).

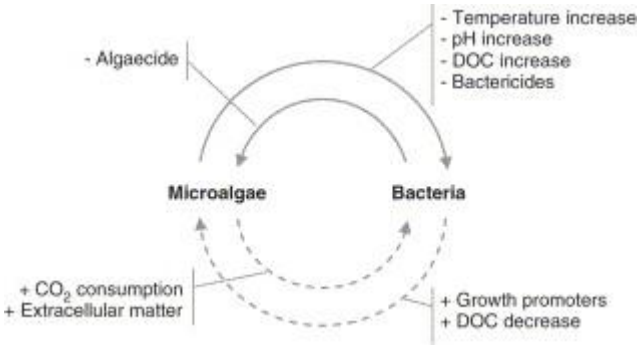

Figure 1 Positive (dashed line) and negative (plain line) interactions between microalgae and bacteria (Munez et al., 2006).

Removal of toxic compounds

A lot of algal species are used in phytodepuration processes and they are able to remove some harmful compounds from the environment, if these compounds are present in relevant quantity.
Table 1 shows compounds, that algae are able to remove; these information derived from the work of Munoz et al. (2006).

Application	Comment	References
BOD removal	Microalgae release of 1.5–1.92 kg O_2 on kg of microalgae produced during photoautotrophic growth and oxygenation rates of 0.48–1.85 kg O_2 m^{-3} d^{-1} have been reported in pilot-scale ponds or lab-scale tank photobioreactors treating municipal or artificially contaminated wastewater	Grobbelaar et al., 1988; Martinez Sancho et al., 1993; McGriff and McKinney, 1972; Muñoz et al., 2004; Oswald, 1988
Nutrient removal	Microalgae assimilate a significant amount of nutrients, because they require high amounts of nitrogen and phosphorous for proteins (45–60% of microalgae dry weight), nucleic acids and phospholipids synthesis. Nutrient removal can also be further increased by NH_3 stripping or P precipitation due to the raise in the pH associated with photosynthesis.	Laliberté et al., 1994; Oswald, 2003; McGriff and McKinney, 1972; Nurdogan and Oswald, 1995; Vollenweider, 1985
Heavy metal removal	Photosynthetic microorganisms can accumulate heavy metals by physical adsorption, ion exchange and chemical sorption, covalent bonding, surface precipitation, redox reactions or crystallization on the cell surface. Active uptake that often involves the transport of the metals into the cell interior is often a defensive tool to avoid poisoning or it serves to accumulate essential trace elements. Microalgae can also release extracellular metabolites, that are capable of chelating metal ions. Finally, the increase in pH associated with microalgae growth can enhance heavy metal precipitation	Chojnacka et al., 2005 Kaplan et al., 1995; Kaplan et al., 1987; Rose et al., 1998; Travieso et al., 1996; Van Hille et al., 1999. Wilde and Benemann, 1993; Yu and Wang, 2004
Pathogen removal	Microalgae enhance the deactivation of pathogens by raising the pH value, the temperature and the dissolved oxygen concentration of the treated effluent	Aiba, 1982; Mallick, 2002; Mezrioui et al., 1994 ; Robinson, 1998; Schumacher et al., 2003
Heterotrophic pollutant removal	Certain green microalgae and cyanobacteria are able to use toxic recalcitrant compounds as carbon, nitrogen, sulphur or phosphorous source	Semple et al., 1999; Subaramaniana and Uma, 1997
Biogas production	CH_4 production from the anaerobic digestion of algal–bacterial biomass allows substantial economical savings	Eisenberg et al., 1981; Oswald, 1976

Table 1: Reported studies on harmful compounds removal by microalgae taken from Munez et al. (2006).

The process parameters such as pH, temperature etc change in function of the algal species.

In **Table 2,** the algal species that can be used to remove toxic compounds are reported:

Species	Wastewater	Plants	references
Chlorella vulgaris	Domestic water	High rate algal pond	Oswald ,1991
Scenedesmus spp.	Domestic water	High rate algal pond	Oswald ,1991
Scenedesmus acutus	Wastewater	Column Reactor	Travieso et al 1999
Scenedesmus obliquus	Wastewater	Rotatory biofilms	Travieso et al, 2002
Homosphaera	Wastewater	Flanks	Fukami et al ,1988
Chlorella pyrenoidosa	Wastewater	Flanks	Khoshmanesh et al., 1996
Chlamydomonas reinhardtii	Wastewater	Polyethylene flasks	Khoshmanesh et al., 1996
Chlorella sarokiniana	Wastewater	Column reactor	Akhtar et al, 2003

Table 2: Main algae species used in phytodepuration processes.

INSTALLATION DESIGN PARAMETERS (POND AND PHOTOBIOREACTOR)

In the following, the main designing characteristics for the two different microalgae culture systems considered, pond and photobioreactor installations, are reported, as gathered from survey in scientific literature.

Culture system: pond

Borowitzka (1999) described different types of culture system for the production of commercial algae, providing a technical structure of these plants. According to this author, culture systems can be classified in **open** and **closed systems** (Borowitzka, 1999). In this report, only information about open systems are reported.

The main types of open-air systems currently in use are:

1. big ponds;
2. tanks;
3. circular ponds;
4. raceway ponds.

The selection of a specific system is influenced by specific requirements, intrinsic properties of the employed alga as well as local climatic conditions and costs of land and water. **Table 3** shows different culture systems types applied in different locations, indicating employed alga species, and information about the size.

Culture system	**Algae species**	**Maximum volume (liters)**	**Extension and/or depth**	**Location**	**Notes**
Tanks	Many species	$1*10^4$		World wide	
Extensive open pond	Dunaliella salina	$1*10^9$	250 ha; 50 cm of depht	Australia	Without mixing
Circular pond with rotating arm	Chlorella spp.	$1.5*10^4$		Taiwan, Japan	Good system
Raceway ponds	Chlorella spp., Spirulina spp., Dunalliellla salina	$3*10^4$	20-30 cm of depht	Japan, Taiwan, USA, Thailand, China, India, Vietnam, Chile, Israel	
Cascade system with baffles	Chlorella spp.	$3*10^4$		Czch Republic, Bulgaria	
Large bags	Many species	$1*10^3$		World wide	Used in aquaculture
Fermenters (heterotrophic)	Chlorella spp. Crypthecodinium cohnii	$>10^3$		Japan, Taiwan, Indonesia, USA	
Two-stage system (indoor closed reactor + out-door paddlewheel ponds)	Haematococus pluvialis	?		USA	
"Caracol" giant spiral shaped	Spirulina (it grows naturally)		3200 m of diameter with a surface area of 900 ha	Mexico	No valid: need of periodical new inoculation of the pond
Pond (similar to the paddle-wheel raceway system)	Chlorella		0.5 ha sloping	Dongara Australia	Plastic lined pond operating in semi-continuous mode

Table 3: Different operating culture systems.

Borowitzka (1999) reports that the **pond depth** (ranging from 20 to 50 cm) needs to be a compromise through:

- the need to provide adequate light to the algal cell;
- the need to maintain an adequate water depth for mixing;
- the need to avoid large changes in ionic composition due to evaporation.

The **mixing** is fundamental and it can be realised in different ways (i.e. paddle-wheel in raceway ponds). The algae are also **CO_2** limited and the addition of CO_2 in large ponds with an extension higher than 25 ha is usually inefficient and not economically sustainable.
The **temperature and the pH** control result to be difficult in a open system and no literature case about pond system reported a monitoring and control system.
In **Table 4**, taken from Borowitzka (1999), properties of different algal culture systems are summarised.

Comparison of the properties of different large-scale algal culture systems

Reactor type	Mixing	Light utilisation efficiency	Temperature control	Gas transfer	Hydrodynamic stress on algae	Species control	Sterility	Scale-up	Reference
Unstirred shallow ponds	Very poor	Poor	None	Poor	Very low	Difficult	None	Very difficult	Borowitzka and Borowitzka, 1989
Tanks	Poor	Very poor	None	Poor	Very low	Difficult	None	Very difficult	Fox, 1983
Circular stirred ponds	Fair	Fair–good	None	Poor	Low	Difficult	None	Very difficult	Tamiya, 1957; Stengel, 1970; Soeder, 1981
Paddle-wheel Raceway Ponds	Fair-good	Fair–good	None	Poor	Low	Difficult	None	Very difficult	Weissman and Goebel, 1987; Oswald, 1988
Stirred Tank reactor (internal or external lighting)	Largely uniform	Fair–good	Excellent	Low–high	High	Easy	Easily achievable	Difficult	Pohl et al., 1988
Air-Lift reactor	Generally uniform	Good	Excellent	High	Low	Easy	Easily achievable	Difficult	Jüttner, 1977
Bag Culture	Variable	Fair–good	Good (indoors)	Low–high	Low	Easy	Easily achievable	Difficult	Baynes et al., 1979
Flat-Plate reactor	Uniform	Excellent	Excellent	High	Low–high	Easy	Achievable	Difficult	Hu et al., 1996; Tredici and Zitelli, 1997
Tubular reactor (Serpentine type)	Uniform	Excellent	Excellent	Low–high	Low–high	Easy	Achievable	Reasonable	Richmond et al., 1993; Torzillo, 1997
Tubular Reactor (Biocoil type)	Uniform	Excellent	Excellent	Low–high	Low–high	Easy	Achievable	Easy	Borowitzka, 1996

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Table 4: Comparison of properties of different large-scale algal culture systems.

Craggs et al. (2004) gave information about the **depth** for conventional ponds, ranging from 0.2 to 0.6 m for shallow pond, and from 1.0 to 1.5 m for deeper ponds. They studied phytodepuration by Scenedesmus in high rate pond at pilot scale and they tested different physical-chemical conditions, such as temperature (from 18°C to 33°C), pH (from 8 to 9.3) and dissolved oxygen (DO) (from 0 g/m^3 to 24 g/m^3) in relation to solar irradiance from 0 W/m^2 to 1200 W/m^2). This work showed that, when pH was higher than 9 and DO under super-saturation condition, it is possible to have the disinfection of wastewater, with an enhanced E. coli (bacteria) death.
In Tredici et al. (1992), they showed different ponds system installed in Italy, used for algal biomass production, describing different pond shapes:

- circular pond;
- raceway pond;
- inclined surface (3.5 cm) with regularly arranged trapezoidal embossments;
- rectangular pond stirred with the mixing board.

In this work, Tredici et al. showed that possible troubles in using open ponds are the temperature flotation according to the type of algae and the evaporation during the summer. The algal species used were:

- Chlorella
- Scenedesmus
- Coelastrum
- Tetraselmis
- Spirulina paltensis and Spirulina maxima in the summer they also live at temperature major 35°C

Culture system: photobioreactor

The available photobioreactor configurations are numerous (Lee, 1986; Tredici and Materassi, 1992; Borowitzka, 1996; Pulz and Scheinbenbogen, 1998), but they usually can be classified into two types: either tubular devices or flat panels. Then, these systems can be also categorized according to the orientation of tubes or panels, the mechanism for culture circulating, the light supplying systems, the type of gas exchange system, the arrangement of the individual growth units, the materials employed for construction and the methods for nutrient supplying.
In **Table 5** different tubular photobioreactor characteristics are shown, focusing on the algal species employed, the main parameters useful to the biomass growth (temperature and pH), the adopted mechanism of circulation, the methods of light supply and the maximum illumination flux provided, the system of CO_2 supply and the materials employed in the construction of the reactors.
In **Table 6**, the main characteristics for flat photobioreactor are summarised, as reported in the following.

Tubular Photobioreactor	Algae	Approximate capacity volume [l]	T [°C]	pH	Circulating mechanism	Light supply methods and illumination flux	CO_2 supply	Construction materials
Indoor	Spirulina sp. and Scenedesmus obliquus	-	30	Determined by a digital pH-meter	Agitation and aeration using air from a compressor and a sintered sparger	A 12 h dark/light photoperiod with 3200 lux of illumination provided by a 40 W daylight-type fluorescent lamp	CO_2 added to the air at a rate of 0.3 vvm for 15 min every 2 h during the 12 h light period	
	Chlorella sorokiniana	58	Controlled by sprinkling the reactor surface with tap water			Natural illumination by solar light energy	CO_2 added to the air at a rate of 0.25 vvm	
Outdoor: consisting of 4 m tall airlift section with a degasser zone	P. Tricornutum	200			Airlift device	Solar illumination		Plexiglass
Semi-continuous	Chlorella sp.		26±1	Directly determined by an ISFET pH-meter KS723		Continuous cool white fluorescent illumination at the intensity of 300 $\mu mol_{photone}\ m^{-2}\ s^{-1}$ measured at the surface of the photobioreactor using a Basic Quantum Meter	Air of different CO_2 concentration produce mixing air and pure CO_2 at a rate of 0.25 vvm	
Channel: Chambers interconnected by means of horizontal baffles attached alternately to the front and the back of the larger flat faces of the reactor	Chlorella vulgaris	3	Controlled by circulating cooling water through a transparent jacket located at the front of the reactor	6.8±1 measured by a sensor placed in the upper part of the reactor and controlled by injecting CO_2 by demand	By a peristaltic pump	Illumination from the both sides of the tubes with 4 Osram Fluora lamps at the intensity of 120 $\mu E\ m^{-2}\ s^{-1}$	CO_2 added to the air at a rate of 0.45 vvm	plexiglass

Table 5: Tubular photobioreactor systems.

Flat panel Photobioreactor	Algae	Approximate capacity volume [l]	T [°C]	Circulating mechanism	Light supply methods and illumination flux	CO_2 supply	Construction materials
Unshaped disposable bag located between two irons frames		250	Controlled by a heat exchanger consisting of 42 m long, 0.025 m diameter stainless steel tubes located 0.05 m above the gas sparger inside the bag	Gas sparger (20 mm PVC tube with 1 mm holes every 3 cm)	Solar illumination measured using a LICOR 200 sensor connected to a data acquisition board in the range of 13 – 30 MJ/m^2 d		plastic
Airlift	Chlorella vulgaris	3	Controlled by circulating cooling water through a transparent jacket located at the front of the reactor	Airlift device	Illumination from both sides of the tubes with 4 Osram Fluora lamps at the intensity of 120 µE m^{-2} s^{-1}	CO_2 added to the air at a rate of 0.45 vvm	Plexiglass
Vertical flat plate	Nannochloropsis sp.		Controlled by cooling by water-spray: cooling is accomplished by having sprinklers set 40 cm apart in a plastic tube extending across the upper part of the reactor. The sprayed water runs down the front and the back panels and it is collected troughs. The sprayed water is recycled, passing through a ventilated water column which cools water to 20 – 22 °C.	Mixing is affected by letting compressed air stream out of a perforated plastic tube extending all across the bottom of the reactor	Continuous illumination from both sides totalling 300 µE m^{-2} s^{-1} white fluorescent light	Provided by compressed air	

Table 6: Flat panel photobioreactor systems.

The most scalable designs correspond to horizontal or helical tubular systems, as well as combinations of vertical flat panels and bubble columns, and so these types of photobioreactors have attracted most interest.
Flat panel photobioreactors feature important advantages for mass production of photoautotrophic microorganisms and may become a standard reactor type for the mass production of several algal species (Sierra et al., 2007). The construction of flat plate reactors dates back to the early 1950s (Burlew, 1953). Samon and Leduy (1985) used vertically translucent flat plates, illuminated on both sides and stirred by aeration; Tredici and Materassi developed this idea (Tredici et al., 1991, Tredici and Materassi, 1992) proposing a rigid alveoplar panel; Pulz et al. (1995) used flat panels with inner walls arranged to promote an ordered horizontal culture flow that was forced by a mechanical pump; the research of Hu and Richmond (Hu and Richmond, 1996; Hu et al., 1998) resulted in a type of flat plate reactor made of glass sheets, glued together with silicon rubber to make flat vessels. Recently a new design of vertical flat panel photobioreactor consisting of a plastic bag located between two irons frames has been proposed (Tredici and Rodolfi, 2004), bringing a substantial cost reduction to this type of reactors.
Some environmental factors, e.g. temperature and mineral nutrients supply, are relatively easily controlled, but others such as the supply of solar radiation are more difficult to regulate.
Availability and intensity of light are the major factors controlling productivity of photosynthetic cultures (Lee and Low, 1992; Pulz and Scheinbenbogen, 1998).
Outdoor cultures are subjected to cyclic changes in irradiance levels, distinguishing two cycles with substantially different times: a relatively long daily cycle and a yet longer cycle based on the change of seasons during the year.
The radiation incident on the surface of a photobioreactor consists of direct sunlight, reflected radiation from the surroundings and diffuse radiation due to particulate matter in the atmosphere. The incident light level on an outdoor reactor is a function of time, the geographic location of the reactor and environmental factors (Incropera and Thomas, 1978).
The light spectrum shows some deviations from sunlight and only a small number of colours are available. For this reason in the last few years light emitting diodes (LEDs) have become increasingly interesting for use in laboratory photobioreactors (Sastre et al., 2007).
Carbon dioxide is the usual carbon source for photosynthetic culture of microalgae. Carbon dioxide is typically supplied by continuous or intermittent injection of the gas at the beginning of a tubular solar receiver. As the carbon is consumed, oxygen is ultimately produced by photolysis of water. The generated oxygen is released into the culture medium. The concentration of carbon dioxide reflected in the culture pH changes (Livansky and Bartos, 1986).
Furthermore one of the most understudied methods of CO_2 reduction is the use of microalgae that convert CO_2 form a point source into biomass. Microalgae use CO_2 efficiently because they can grow rapidly and can be rapidly incorporated into engineered systems, such as photobioreactors (Carvalho et al., 2006; Lee and Lee, 2003; Suh and Lee, 2003).

(2) Definition of the climatic and environmental variables affecting the phytodepuration technique with microalgae in the territory of Varese Ligure.

The information obtained in the previous section (1) have been used to define critical points and possible solutions about phytodepuration technique applied in Varese Ligure territory. **Table 7** summarises the most relevant environmental parameters that influence the process of phytodepuration by microalgae. In column B, possible critical points in relation to the ambient and the climatic conditions of Varese Ligure (column D) are reported. In column C, some possible solutions are proposed in order to maintain the system in function; these solutions have been taken by the scientific literature.

A	B	C	D
Parameter	**Critical point and evidences**	**Possible solution**	**Varese Ligure**
Temperature	Two seasonal periods have been identified.		Average annual temperature 9-14°C
	1. November –March	It is necessary to increase the temperature in the culture system until 10 °C: **it can be useful to have a greenhouse**, in which to place the algal culture system.	The average temperature in the winter are nearing 0°C÷ -1°C
	2. April - October	No solution is required because temperatures are in the optimal range for the algal process	Temperate climate
Light	Light greatly influences the algal growth: **the optimal range of incident irradiance is from 800 to 1400 µE/m²s.**		Latitude: **44°22'38"28 N** Longitude: **09°35'42"72 E**
	Photo-inhibition troubles: **It is possible to have photo-inhibition effects about 1630 µE/m²s.** The peak light level may exceed 2000 µE/m²s , which is several times above the saturation irradiance level (Molina et al., 1999).	Summer period: it is necessary to screen the light in the central hours of the day.	
Nutrients	The algal cultures can have several problems	**Optimum relation** **1:10** Phosphorus: Nitrogen	The wastewater quality coming from the primary

	with high concentration of nutrients (nitrogen and/or phosphorus), while with low nutrient concentration the algal culture is able to grow	(from scientific literature). **<u>Classical Redfield ratio</u>** **1:16** P:N (from Voltolina et al., 1998). **<u>Low concentration of P</u>** **1:15** P:N **1:30** P:N**(from others scientific works)** **<u>Low concentration of N</u>** **1:5** P:N (from others scientific works)	settler of the plant of **San Pietro Vara** of Varese Ligure is in these ranges : $N\text{-}NH_4$= 9.8-68mg/l $N\text{-}NO_3$= 0.4-0.5 mg/l $P\text{-}PO_4$= 0.30-2.70mg/l Relation: 1:30 P:N
O_2 and CO_2 supply	It is necessary to supply CO_2 to the algal system	To supply CO_2	Probably the plants: photobioreactor and pond will build open system.
	In the open algal system (pond) CO_2 comes from the air;	It is possible to increase the gas exchange moving the algal culture.	
	In the closed system (photobioreactor) the CO_2 is artificially supplied	It is possible to build the capture and sequestration systems of CO_2	
pH	pH is continuously monitored. It is maintained in the optimal range. pH range is dissimilar to different algal species. But at pH >8 phosphorous precipitates and ammonium nitrogen gasifies, so the nutrients are not available to the algal biomass.		

Table 7: Environmental parameters, critical points and solutions that can influence the algal phytodepuration in the territory of Varese Ligure.

Other consideration coming from the close examination of the scientific literature are that it is always necessary to have algal inoculums, because the unfavourable ambient conditions, <u>the **predators**</u> such as Protozoa and moreover <u>the **competition**</u> between algae and bacteria, can weigh on the growth of the algal population. Moreover, in the open pond system, there is always the possibility of a continuous contamination by other algae species and predators organisms of the algae.

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