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EFFECT OF LIGHT INTENSITY INTRODUCED THROUGH OPTICAL FIBRES ON SOIL REDOX STATUS AND GASES EVOLUTION

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Abstract. This paper proposes a new solution for improving oxygenation state of anaerobic medium by means of optical fibres. Visible light (400-750 nm) of varying intensity (811-4866 lx) was introduced through optical fibres to an anaerobic medium (*Eutric Fluvisol*) for 10 days, which could activate phototrophic microorganisms producing oxygen, and indirectly change the redox potential (Eh) and the gas composition formed during the incubation period. Control showed a significant decrease of Eh from the initial level of 320.8 mV to 50.6 mV at the end of incubation. Illumination caused buffering of Eh of tested medium. In these reactors ΔEh was 130.7 mV for 811 lx, 80.7 mV for 4866 lx and the most advantageous combination was 2433 lx where ΔEh was only 30.2 mV. In the illuminated units maximal concentration of oxygen was ~2.5% (811 lx), ~6% (2433 lx) and ~5.1% (4866 lx). The formation of N_2 at about 20% for the combination of 2433 lx and 4866 lx, and about 15% for 811 lx was also observed. Respiration activity of phototrophs revealed a high level of CO_2 1.3% (811 lx), while the stronger illuminations led to CO_2 concentrations of only 0.5% which was connected with intense binding of this gas in the photosynthesis process. Obtained results emphasise the key role of light in anaerobic soil medium. Oxygen produced by the activity of phototrophs may indirectly affect the redox state by Eh buffering and thus prevent anaerobiosis. It also affects gas formation, which may have positive environmental consequences.

Keywords: optical fibres, anaerobiosis, soil suspension, redox potential, phototrophs

INTRODUCTION

During evolution nearly all life forms have been exposed to electromagnetic radiation emitted by the sun which emits light in a wide wavelength range. Photosynthetically active radiation (PAR) reaching the Earth surface is only a small fraction of this range and it is a major parameter controlling many biological and physical processes related to the evolution of plant canopies, agricultural and en-

vironmental fields (Rubio *et al.* 2005). The phototrophic way of life implies the capturing of electromagnetic energy, its conversion into chemical energy, and its use for cellular maintenance and growth (Schlegel *et al*. 2000). On the oxygenic photosynthesis way energy is gained by plants, *Protista* and cyanobacteria (*Oxyphotobacteria*) (Carr and Whitton 1983). The last group of microorganisms has a particular importance for the environment, especially where anaerobic conditions prevail, e.g. in wetlands or landfills (Kewei 2006). Supplying the light energy (400-700 nm) to those places, characterised by highly reductive conditions, may increase the phototrophs activity and may influence the direction of biochemical pathways due to changes of redox state. The development of oxygenic photosynthesis created the modern biosphere, because phototrophs are the only organisms ever to evolve coupled photosystems that harvest electron from water and produce dioxygen as a bioproduct (Carr and Whitton 1983).

Soil is a heterogeneous environment where the aerobic and anaerobic conditions exist side by side. Soil aeration is closely linked with the relation of air-water conditions. This affects the biological activity of soil microorganisms which are very sensitive to oxidation or reduction processes (Gliński and Stępniewski 1985). After soil flooding the pool of gases, especially O_2, rapidly decreases and the time of oxygen depletion in soil depends on the size of oxygen pool and the actual oxygen consumption rate (Stępniewski *et al.* 2005). It is estimated that there is a shift from aerobic to anaerobic conditions when oxygen concentration is less than 1% (Paul and Clark 1996). This process could take even a few hours for the microorganisms to consume all remaining oxygen pool. After oxygen exhaustion (first terminal electron acceptor) microorganisms use alternative electron acceptors resulting in a cascade of reactions (Ponnamperuma 1972, Banach and Stępniewska 2011).

The aim of this work was to show the importance and effect of visible light, introduced through optical fibres to soil medium at varying intensity, on the inhibition of anaerobic processes.

MATERIAL AND METHODS

Soil material (*Eutric Fluvisol*) was collected from the depth of 0-20 cm in Małopolski Gorge of the Vistula River (51°10’05’’N 21°47’15’’) in Poland. The main soil characteristics are presented in Table 1.

The experiment was conducted on soil suspension prepared from the collected samples (soil to water ratio of 1:2.5) placed in bioreactors. Autoclaved soil suspension (121ºC, 103 kPa for one hour) served as a control (no illumination) and 3 illuminated treatments were prepared using a combination of different light intensities of 811, 2433 and 4866 lx. Next, the photoreactors were washed with helium

in order to remove oxygen and isolated from daylight by covering with aluminium foil prior to the incubation.

Table 1. The main soil characteristics

Depth (cm)	Particle size distribution (% of fraction in mm)					
	1-0.1	0.1-0.05	0.05-0.02	0.02-0.005	0.005-0.002	<0.002
0-20	30	34	21	6	4	5

Finally, the reactors were installed on magnetic stirrers (mixing speed of 300 rpm) which aimed to provide homogenous soil suspension during 10 days of incubation at 25^{o}C (Fig. 1).

Optical fibres (PMMA) emitting photosynthetically active radiation (PAR) quantified as 90±10 µmol of photons m^{-2} s^{-1} were used. Spectral output power range was measured with Ocean S200 Fibre Optic Spectrometer.

Analysis of gases liberated to the headspace during the incubation was carried out and tested by the gas chromatography-mass spectrometry techniques under the following conditions: 30 m column Supel-Q, column oven T = 40^{o}C, ion source T = 250^{o}C, injection T = 50^{o}C, helium was the carrier gas flowing at the rate of 40 ml min^{-1} (GCMS-QP 2010 Plus, Shimadzu). Carbon forms in soil suspension were analysed at the beginning and final stage of the experiment (TOC-V CSH, Shimadzu).

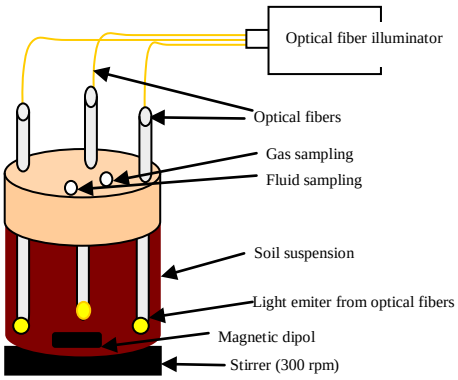

Fig. 1. Scheme of a single photoreactor equipped with optical fibres

Measurements of pH and redox potential (Eh) were performed by potentiometric method, meter pIONeer 65, at resolution 0.1 mV and accuracy ±10^{12} Ohm with combined electrodes (Radiometer Analytical AS).

One-way analysis of variance (ANOVA) was performed to determine the significance of differences between the pairs of means. The differences were statistically significant when P-value was less than 0.05. Multiple Range Test was done (with 95.0% confidence levels) to find out the levels of significance for differences between mean values (at $p<0.005$ and $p<0.01$).

RESULTS AND DISCUSSION

Eh dynamics

The impact of light intensity on the Eh value and formed gases was tested over time. In the control combination a significant decrease in the Eh at the initial level from 320.8 mV to 50.6 mV on the last day of incubation was observed. This result indicates that oxygen and nitrate were not present and the iron and manganese forms were reduced. However, sulphate was stable in the soil without sulphide production, which is toxic to plants and acts as a strong poison of PSII activity in many algae and cyanobacteria (Stal and Moezelaar 1997). Illumination combinations characterised by buffering the redox state and ΔEh were 130.7 mV for 811 lx and 80.7 mV for 4866 lx, and the most advantageous combination was the 2433 lx where ΔEh was only 30.2 mV (Fig. 2). This buffering effect of Eh indicated the presence of O_2.

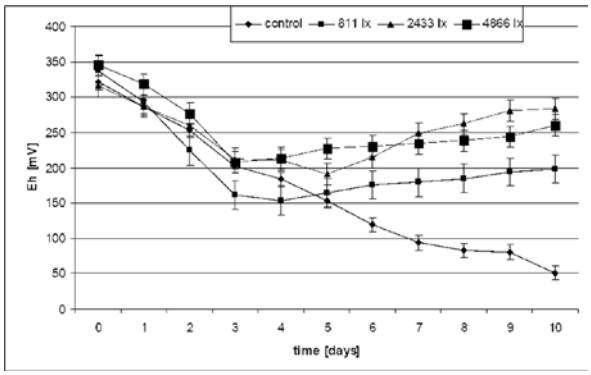

Fig. 2. Eh dynamics in soil suspension equipped with optical fibres and control ($p<0.001$, $n = 144$)

Gas dynamics

During the incubation a continuous monitoring of formed gases was performed. The maximum of oxygen concentration in illuminated reactors was found at the level of 2.5%, 6%, 5.1% for 811 lx, 2433 lx, 4866 lx treatments, respectively (Fig. 3a). The formation of molecular nitrogen at the level of 20% for the combinations of 2433 lx and 4866 lx was observed, while N_2 concentration was at the level of about 15% in combination 811 lx (Fig. 3b). The effect of oxygen and nitrogen presence was not observed in control combination (no illumination).

Based on these data it could be concluded that the intensity of light is a limiting factor for the photosynthesis of phototrophic soil microorganisms, as is the case of higher plants (Prabodh *et al.* 1997, Vas and Sharma 2009) and e.g. water algae (Shengzhang *et al.* 2011). The intensity of light of 2433 lx seemed to be optimal, with higher levels of O_2 and N_2. There was no significant increase of oxygen concentration, the provider of the intensification of the oxygenic photosynthesis, at higher intensity. However, the lowest tested light intensity (811 lx) was not sufficient to activate the growth of phototrophs present in the suspension

and the effect of oxygen concentration was negligible (Fig. 4). Respiration activity of microorganisms (phototrophs) was revealed a high level of CO_2 of 1.3% in the case of the combination of 811 lx, while the stronger illuminations were characterised by CO_2 concentration of only 0.5%, which was connected with intense binding of this gas in the photosynthesis process (Fig. 3c).

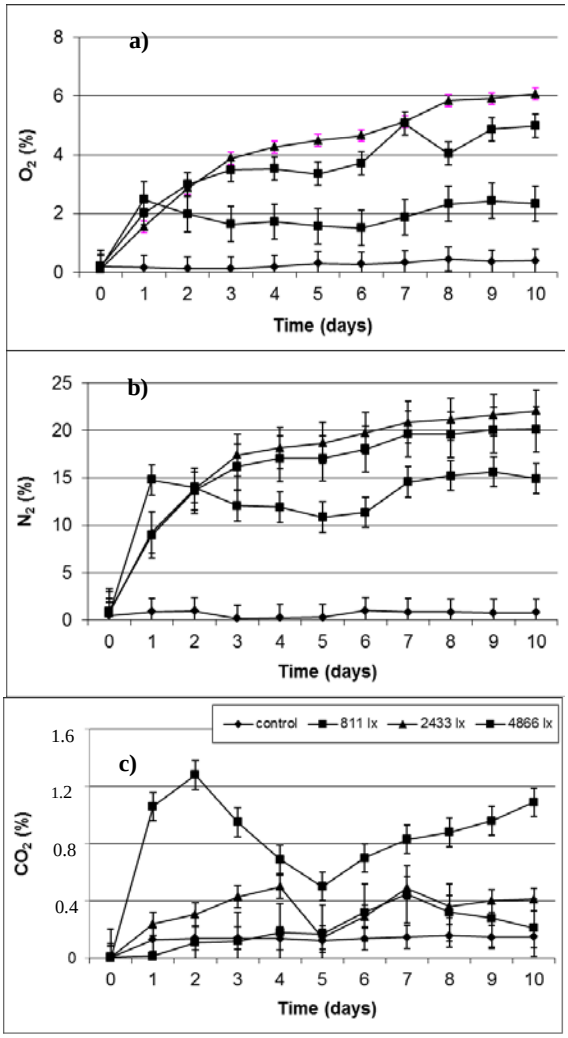

Fig. 3. Dynamics of concentrations of a) oxygen, b) nitrogen, c) carbon dioxide in the treatments ($p<0.001$, $n = 144$)

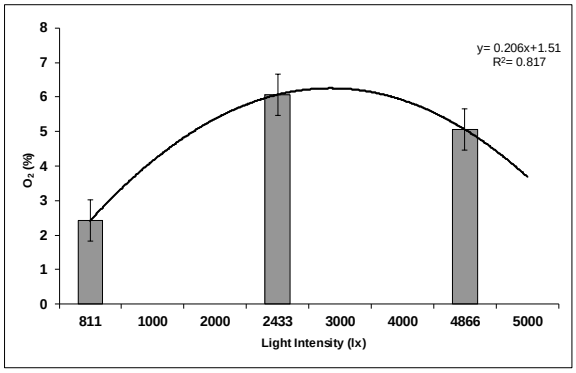

Fig. 4. The dependence of oxygen concentration on light intensity ($p<0.001$, $n = 144$). Error bars indicate the standard error

Redox potential in soil solution is a result of many factors, mainly the amount of dissolved organic oxygen and its consumption by organisms. It is estimated that the change from aerobic to anaerobic conditions occurs when oxygen concentration falls below 1% (Paul and Clark 2000) and when Eh drops to 300 mV which corresponds to the reduction of iron oxides (Strumm and Morgan 1996). Control treatment had anaerobic conditions and autoclaved soil did not show microbial activity, which agrees with low Eh values (50 mV) and gases composition in the headspace. The observed slight increase (less than 1%) of gases concentrations (oxygen, nitrogen and carbon dioxide) is related to the chemical transformations of organic and mineral soil components (e.g. oxidation of reduced forms of iron, manganese, decomposition of carbonates etc.). The results of Majewska *et al.* (2006) and Serrasolsas *et al.* (1995) confirm that sterilisation processes completely inhibit soil microbial activity, however, as reported by Carter *et al.* (2007), some soil enzymes remain stable under such extreme conditions, e.g. phosphodiesterase – an important enzyme in the phosphorus cycle in nature.

Table 2. Forms of nitrogen on the initial and final days of incubation (mg l^{-1})

Treatments	$N\text{-}NO_3$		$N\text{-}NO_2$		$N\text{-}NH_4$		pH H_2O		Corg (%)	
	1*	2*	1*	2*	1*	2*	1*	2*	1*	2*
Control	20.32	18.01	0.042	0.017	4.784	4.266	7.45	7.68	9.27	11.48
811 lx	20.01	12.74	0.037	0.022	5.017	2.142	7.45	7.15	9.27	12.22
2433 lx	20.12	9.24	0.042	0.005	4.923	1.519	7.45	7.01	9.27	12.83
4866 lx	20.52	10.30	0.034	0.012	4.741	2.012	7.45	6.93	9.27	12.66

1*– initial, 2* – final.

The introduction of additional energy in the form of radiation quanta from visible light could result in the shift from anaerobic to aerobic conditions. In illuminated treatments the formation of molecular oxygen was observed, indicating the activation of soil oxygenic phototrophs and the redox potential in this treatments was stabilised on a level corresponding to moderately aerobic state. Stabilisation of Eh at a pretty high level is related to oxygen present in the headspace and probably relatively high content of nitrates is soil suspension. According to Patrick (1978), the reduction of nitrate occurs at Eh values between 300 and 200 mV, the denitrification rate depends on nitrate concentration and is slower in acid soils than in neutral pH (Kemmitt *et al.* 2006). Denitrification is a special kind of nitrate respiration in which the end-products are gaseous forms such as N_2, N_2O and NO returned to the atmosphere. It is used by many groups of soil microorganisms, both heterotrophic (*Pseudomonas, Bacillus, Serratio, Vibrio*) and autotrophic (*Thiobacillus denitrificants, Micrococcus denitrificans, Fusarium oxysporum, Fusarium solani*) (Firestone 1982, Wrage *et al.* 2001). This process is strictly anaerobic but it can be observed even when soil air contains some oxygen, because anoxic microsites exist inside soil aggregates. Another process which is responsible for nitrogen production is anammox. As denitrification, the anammox process closes the biogeochemical nitrogen cycle and it may be an important process for the removal of nitrogen from ecosystems (Francis *et al.* 2007, Kuenen 2008).

CONCLUSIONS

The light energy, delivered through the optical fibres to soil suspension at different intensities of 811, 2433 and 4866 lx, showed that:

1. Phototrophs activity depends on the light intensity, as statistically significant differences were shown for the combination of 811 lx and 2433 lx.
2. There is a stabilization of Eh in all illuminated treatments, according to O_2 formation and relatively high content of nitrates.
3. Low concentration of CO_2 in combinations of 2433 lx and 4866 lx is connected with the intense binding of this gas in photosynthesis.
4. Prevention of anaerobiosis is connected with illumination of soil suspension.

REFERENCES

Banach A.M., Stępniewska Z., 2011. The role of redox conditions on soil nutrients availability. Soil Nutrients, ISBN 978-1-61324-785-3, Nova Science Publishers.

Carr N.G., Whitton B. A., 1983. The biology of cyanobacteria. Berkeley and Los Angeles.

Carter D.A., Yellowlees D., Tibbett M., 2007. Autoclaving kills soil microbes yet soil enzymes remain active. Pedobiologia, vol. 51, 4, 295-299.

Firestone M.K., 1982. Biological denitrification. In: Stevenson F. J. (Ed.) Nitrogen in agricultural soils. Agronomy, 22, 289-326.

Francis C.A., Beman J.M., Kuypers M.M.M., 2007. New processes and players in the nitrogen cycle: the microbial ecology of anaerobic and archaeal ammonia oxidation. ISME J. 1: 19-27

Gliński J., Stępniewski W., 1985. Soil Aeration and Its Role for Plants. CRC Press Inc. Boca Raton, Florida.

Kemmitt S. J., Wright D., Goulding K. W. T., Jones D. L., 2006. pH regulation of carbon and nitrogen dynamics in two agricultural soils. Soil Biology and Biotechemistry 38, 898-911.

Kewei Y., Faulkner S.P., Patric W.H. Jr., 2006. Redox potential characterization and soil greenhouse gas concentration across a hydrological gradient in a Gulf coast forest Chemosphere, 62, 905-914.

Kuenen J.G., 2008. Anammox bacteria: from discovery to application. Nat. Rev. Microbiol., 6, 320-326.

Majewska M., Kurek E., Szlachetka D., 2006. Microbial activity - Factor increasing retention of Cd addend to soil. Polish J. Environ. Stud. Vol. 15, No. 2a, 127-134.

Patrick W. H., 1978. Critique of "Measurement and prediction of anaerobiosis is soils" by Stolzy, L. M. and Fluhler H., in Nitrogen in Environment, vol. 1. New York, 449.

Paul E.A., Clarc F.E., 2000. Microbiology and soil biochemistry, UMCS, Lublin, Poland.

Ponnamperuma F.N., 1972. The chemistry of submerged soils. Adv. Agron., 24, 29-33.

Prabodh K.T., Pravendra N., Prafullachandra V.S., 1997. Photoinhibition without net loss of photosystem II components in Populus deltoids. J. Biosci., Vol. 22, No 3, 345-355.

Rubio M.A., Lo´pez G., Tovar J., Pozo D., Batlles F.J., 2005. The use of satellite measurements to estimate photosynthetically active radiation. Physics and Chemistry of the Earth 30, 159–164.

Schlegel, R. A., M. K. Callahan and P. Williamson. 2000. The central role of phosphatidylserine in the phagocytosis of apoptotic thymocytes. Ann. N. Y. Acad. Sci. 926:217-225.

Serrasolsas I., Khanna P.K., 1995. Changes in heated and autoclaved forest soils of S. E. Australia. Phosphorus and phosphatase activity. Biogeochemistry 29, 25-41. Schlegel H. G., 2000. Mikrobiologia ogólna. PWN.

Shengzhang X., Zhenfeng S., Wei C. Growth of Spirulina platensis enhanced under intermittent illumination. 2011. Journal of Biotechnology 151, 271–277.

Stal L.J., Moezelaar R., 1997. Fermentation in cyanobacteria. FEMS Microbiology Reviews, 21, 179-211.

Stępniewski W, Stępniewska Z., Bennicelli R. P., Gliński J., 2005. Oxygenology in outline. EU 5th Framework Program QLAM-2001-00428, Lublin.

Strumm W., Morgan J.J., 1970. Aquatic Chemistry, Wiley Interscience, 33-382.

Vas J., Sharma P.K., 2009. Photoinhibition and photosynthetic acclimation of rice (Oryza sativa L. cv Jyothi) plants grown under different light intensities and photoinhibited under field conditions. Indian Journal of Biochemistry and Biophysics, vol. 46, 253-260.

Wrage N., Velthof G.L., van Beusichem M. L., Oenema O., 2001. Role of nitrifier denitrification in production of nitrous oxide. Soil biology and Biochemistry, 33, 1723-1732.

WPŁYW NATĘŻENIA ŚWIATŁA DOPROWADZONEGO POPRZEZ ŚWIATŁOWODY NA STAN OKSYDOREDUKCYJNY ZAWIESINY GLEBOWEJ I FORMOWANE GAZY

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S t r e s z c z e n i e . W niniejszej pracy zaproponowano nowe rozwiązanie w celu poprawienia natlenienia podłoża za pomocą światłowodów. Światło widzialne (400-750 nm) o różnym natężeniu (811-4866 lx) wprowadzone poprzez światłowody do miejsc objętych stanem anaerobiozy (Eutric Fluvisol) na 10 dni co może aktywować fototrofy, produkujące tlen, a pośrednio wpłynąć na zmianę potencjału redoks (Eh) oraz formowane gazów w trakcie inkubacji. W kombinacji kontrolnej zaobserwowano znaczący spadek wartości Eh z poziomu początkowego 320,8 mV do 50,6 mV w ostatnim dniu inkubacji. Kombinacje oświetlone charakteryzowały się buforowaniem stanu oksydoredukcyjnego. W reaktorach tych ΔEh wynosiło odpowiednio: 130,7 mV dla 811 lx, 80,7 mV dla 4866lx. Najkorzystniejsza okazała się kombinacja 2433 lx, gdzie ΔEh wynosiła zaledwie 30,2 mV. W reaktorach oświetlonych maksymalne stężenie tlenu wynosiło odpowiednio: ~2,5% (811 lx), ~6% (2433 lx) oraz ~5,1% (4866 lx). Zaobserwowano również formowanie azotu cząsteczkowego na poziomie około 20% w przypadku kombinacji 2433 lx i 4866 lx oraz około 15% w przypadku 811 lx. Aktywność respiracyjna fototrofów ujawnia się w wysokim poziomie CO_2 1,3% w przypadku kombinacji 811 lx, podczas gdy silniejsze iluminacje charakteryzowały się stężeniem CO_2 zaledwie 0,5% ze względu na intensywne wiązanie tego gazu w procesie fotosyntezy. Uzyskane wyniki podkreślają kluczową rolę światła w ośrodku glebowym, w którym panują warunki beztlenowe. Tlen produkowany w wyniku aktywności fototrofów, pośrednio może wpływać na buforowanie stanu redoks i tym samym zapobiegać anaerobiozie. Znamiennym jest również efekt formowania gazów o znacznie mniejszej szkodliwości dla środowiska.

S ł o w a k l u c z o w e : światłowody, anaerobioza, zawiesina glebowa, potencjał oksydoredukcyjny, fototrofy