Low-cost integrated sensors chip for environmental monitoring
(環境計測のための低コスト集積型センサチップ)

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By this study, the challenges of frequent environmental monitoring have been overcome by developing and designing a low-cost and simple optical integrated sensors chip which has the capability to effect-free monitoring of three important water quality parameters like dissolved oxygen (DO), biochemical oxygen demand (BOD) and Eco-Toxicity (E-Tox) simultaneously based on the sensitive fluorescence detection of ruthenium complex. Fluorescence as an optical property originates when ruthenium complex absorbs light of a shorter wavelength (447 nm) and emits light of a longer wavelength (604 nm) and the emitted fluorescence intensity (FI) always depends on the presence of oxygen molecules. The more of oxygen, the more of reduction of FI because the oxygen molecules act as the quencher of FI. Since, FI and oxygen bear a good relationship, by perceiving this concept, BOD and E-Tox biosensors were developed by immobilizing baker's yeasts (*Saccharomyces cerevisiae*) and green microalgae (*Pseudokirchneriella subcapitata*) incorporating with ruthenium complex based DO sensor chip where oxygen consumption due to respiration activity by yeasts in presence of organic substances and inhibition of oxygen generation rate (%) by photosynthetic activity of green microalgae in presence of chemicals (pesticides) were effectively investigated by forming sensing protocols.

To reach the noble goal, several features such as DO sensor (array and film) types, elimination of effects caused by environmental samples, suitable immobilization technique of yeasts and microalgae, simple chip fabrication, sensitivity in real environmental samples etc. were evaluated throughout in this study.

Firstly, DO sensors chip were developed and tested in laboratory prepared distilled water (DW) as well as in environmental samples like river water (RW), coastal sea water (CSW) by controlling DO for assessing the sensitivity. Not equal sensitivity in all types of sample was observed and hence the effects (45-50%) especially obtained by RW have been completely eliminated by shielding the oxygen sensing film (OSF) with polyethylene polypropylene (PE-PP) film which only permit to enter oxygen into it but no other substances.

Then BOD biosensor chip was developed by immobilizing yeasts within only polyvinyl alcohol-strylpyridinium (PVA-SbQ) matrix (called biofilm) and attaching the biofilm onto previously developed PE-PP film shielded DO sensor chip (film type). Sensitivity of BOD biosensor using BOD standard solution glucose glutamic acid (GGA) (150 mg/L glucose and 150 mg/L glutamic acid equals to 220 mg/L BOD) was investigated and found good responses where the linear detection range was up to 20 mg/L GGA. The another type biofilm was also prepared by immobilizing yeasts with mixed cellulose ester (MCE) filter membrane and 8% PVA-SbQ matrix but poor sensitivity was observed compare to only PVA-SbQ matrix based biofilm.

Furthermore, the E-Tox biosensor chip was constructed by immobilizing green microalgae within black MCE and Omnipore® filter membranes as well as within only PVA-SbQ matrix and incorporating the biofilms with the DO sensor chip. The filter membranes based biofilm was incorporated with microarray type DO sensor chip and tested six pesticides (diuron, atrazine, simazine, simetryn, mefenacet and pendimethalin) and found very good sensitivity as of dose-response inhibition of photosynthetic activity or inhibition of oxygen generation rate (%) by green microalgae to four pesticides (diuron, atrazine, simazine, simetryn) but almost insensitivity was attained to mefenacet and pendimethalin pesticides. Besides, by using PVA-SbQ matrix based biofilm onto PE-PP film shielded film type DO sensor chip, diuron was also tested and found all most equal sensitivity to the filter membranes based biosensor.

Finally, three sensors were integrated and designed onto a single chip in which the DO sensor chip was protected by PE-PP film and both biofilms (yeasts and green microalgae) were prepared by using only PVA-SbQ immobilizing matrix. The developed integrated sensor performed well in real environmental samples for DO, BOD and E-Tox monitoring.

The present development covers not only low-cost but also other many other advantages including simple measurement technique, real sample applicability, disposable, micro-liter sample volume requirement, single sample for three detections, no chemical discharge into the environment etc. which were the major shortcomings of the conventional or available biosensors (electrochemical). Thus, the proposed technique could lead as the promising and prospective tool towards regular environmental quality monitoring for the protection of our environment.

In the next study, improvement of accuracy, suitable designing, portable and automation of the sensor will be conducted. This integrated approach could also be extended in other areas where oxygen monitoring is severely important like cell culture, cellular function monitoring for drug discovery, aquaculture, medical diagnosis etc.