Integration of Water Treatment and Water Analysis Technology
- Introduction of AIST Water Project -

Hiroaki TAO

AIST Shikoku Center, National Institute of Advanced Industrial Science and Technology (AIST)
Today’s Presentation

- Introduction of AIST
- Outline of AIST Water Project
- R&D on Water Measurement Technology
- R&D on Water Treatment Technology
National Institute of Advanced Industrial Science and Technology

AIST Tsukuba
Work Force

Composition of research staff by research field

- Environment and Energy: 25%
- Life Science and Biotechnology: 17%
- Information Technology and Electronics: 17%
- Nanotechnology, Materials, and Manufacturing: 15%
- Metrology and Measurement Science: 16%
- Geological Survey and Applied Geoscience: 10%

Employees

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Researchers</td>
<td>2,281 [80]</td>
</tr>
<tr>
<td>- Permanent</td>
<td>2,010</td>
</tr>
<tr>
<td>- Fixed term</td>
<td>271</td>
</tr>
<tr>
<td>Administrative employees</td>
<td>657</td>
</tr>
<tr>
<td>Total number of employees</td>
<td>2,938 [81]</td>
</tr>
<tr>
<td>Executives</td>
<td>13</td>
</tr>
<tr>
<td>Visiting researchers</td>
<td>156</td>
</tr>
<tr>
<td>Postdoctoral researchers</td>
<td>259</td>
</tr>
<tr>
<td>Technical staff</td>
<td>1,602</td>
</tr>
<tr>
<td>Researchers accepted through industry-academia-government partnerships [400 from overseas]</td>
<td></td>
</tr>
<tr>
<td>From companies</td>
<td>Approx. 1,700</td>
</tr>
<tr>
<td>From universities</td>
<td>Approx. 2,000</td>
</tr>
<tr>
<td>From other organizations</td>
<td>Approx. 800</td>
</tr>
</tbody>
</table>

(Note: Total no. of researchers accepted in FY 2012)
Today’s Presentation

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- R&D on Water Treatment Technology
Research Institute for Environmental Management Technology

Research Institute for Innovation in Sustainable Chemistry

Health Research Institute

Biomedical Research Institute

Electronics and Photonics Research Institute

Information Technology Research Institute

Dr. Tao
Director of AIST
Shikoku Center

Over 30 researchers of 6 Research Institutes participate in this AIST Water Project

Global water stress
* WBCSD Water Scenarios to 2025
AIST Technologies

Overcome the water problems by integrating AIST owned technologies listed below.

- Online water quality monitoring technology
- Highly sensitive heavy metals detection technology
- High-speed microbial image acquisition and processing technology
- Microbial separation & identification technology
- Membrane bioreactor (MBR)
- Decomposition & sterilization technology by photocatalyst
- Various materials for adsorption film
- Water data transfer and processing using cloud technology

Global Collaboration

- Promotion of personnel exchanges and joint researches with the National Institutes and universities in Asia
- International cooperation for standardization of water technologies
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Item to be monitored for risk assessment

1. Index for Organic Pollutants (BOD, COD, TOC)
2. Heavy Metals
3. Physiologically Active Substances (Endocrine Disrupting Chemicals, PPCPs)
4. Whole Effluent Toxicity (WET)
5. Microorganism
6. Multidrug Resistance Gene (New Delhi metallo-β-lactamase)
7. · · · ·
Maintenance-free TOC monitor using photochemical reaction

- Generation of oxidative species, ·OH, with UV irradiation (<190 nm)
- Persistent substances such as humic acid are decomposed to CO₂ in less than 1 min
- No hazardous reagent and heating device

**Principle**

Lamp-pass-through reactor

H₂O + hν (<190 nm) → H· + OH·
OH· + Organic matter → CO₂

**Comparison with other methods**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Heater</th>
<th>Detection Limit</th>
<th>Maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>none</td>
<td>6.2 μgC/L</td>
<td>no need</td>
</tr>
<tr>
<td>none</td>
<td>&gt;600°C</td>
<td>48 μgC/L</td>
<td>exchange of catalyst, cleaning of heater</td>
</tr>
<tr>
<td>none</td>
<td>&gt;600°C</td>
<td>~100°C</td>
<td>supply of reagent</td>
</tr>
<tr>
<td>none</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Prototype**

TOC, heavy metals
stripping voltammetry using ECR nano-carbon electrode

**Continuous Monitoring of MBR**

Period: 1 month
Precision: 1.8% for 12 h
Good comparability with catalytic combustion method
Online TOC analyzer using a vacuum UV mercury lamp-pass-through photoreactor

\[
\text{H}_2\text{O} + \text{hv} (<190 \text{ nm}) \rightarrow \text{H} \cdot + \text{OH} \cdot \\
\text{OH} \cdot + \text{Org. Comp.} \rightarrow \text{CO}_2
\]
without oxidizing reagent (\text{K}_2\text{S}_2\text{O}_8)
Dependence of lamp output power on the decomposition of fulvic acid at 2 mgC/L
Bioluminescent assay for endocrine-disrupting chemicals

- Hormone receptor-mimicking bioluminescent probes for endocrine-disrupting chemicals (EDCs)
- High-throughput illumination of the activities of EDCs in wastewater and living subjects
- Superluminescent luciferases for supporting a high-sensitive analysis of EDCs of the probes

**Principle**

- Single-chain bioluminescent probe mimicking hormone receptors, which sense hormones and change the conformation. This change exerts reconstitution of the fragments of a luciferase and specific light emission.

**Superluminescent luciferase**

- Comparison of brightness
  - Existing most bright luciferase
  - Developed luciferase

A multicolor imaging probe set. Green and red colors indicate genomic and nongenomic activities of chemicals, respectively.

**Determination of estrogen-like activities of chemicals**

- Luminescence intensity

**Prototype**


**Ongoing research**

- Application to PPCPs (pharmaceutical & personal care products)
- On-site monitoring
- Evaluation of efficiency of water treatment

**Step toward the practical use**

- Cooperation with several companies

- 100 times brighter than ever existing luciferases
- Half-life period of luminescence is long (20 min)
Assays for endocrine-disrupting chemicals

1. high-cost/prolonged time (3h)
2. unavailable to unknown compounds
Principle of Bioluminescent Probe

**Firefly luciferase (FLuc)**

**ON**
- FLuc-N
- FLuc-C
- Androgen receptor (AR LBD)
- addition of substrate
  - luminescence
    - \( \lambda_{\text{max}} = 610 \text{ nm} \)

**OFF**
- dual partitioning
- LXXLL motif
- enzyme active site

**advantages:**
1. Rapid analysis (3h → 20 min)
2. High signal to noise ratio (50 times)
3. Available both in cell system and in non-cell system (test paper)
Development of Superluminescent Luciferase (ALuc™)

Introduction of mutation into luciferase

-ATG AAG TGA-

200 AA x 20 ⇒ 1/4000
Low success probability

Total synthesis of optimal DNA based on bioinformatics

Artificial Luciferase

ALuc™

Comparison of brightness

existing brightest luciferase  ⇒  superluminescent luciferase

- 100 times brighter than ever existing luciferases
- half-life period of luminescence is long (20 min)

DNA gene tree

new species
Development of portable luminometer

Determination of estrogen-like activities of chemicals to be applied to wastewater and treated water by MBR
Biosensor of Hazardous Compounds, based on Microbial Responses

Existing methods for warning water pollution

- Breeding carp and killifish ➔ Check the death on next day
- Microbial sensor (Fuji Electric • Toshiba in Japan) ➔ Difficult management of biological sensors

Needs

- Immediate warning system for water pollution ➔ on-site sensor
- Evaluation of Individual compound ➔ WET: Whole Effluent Toxicity
- Acute effect ➔ Chronic effect
Whole cell biosensor for ecotoxicity testing using human iPS cells

- Whole cell biosensor with broad substrate responses using human iPS cells
- Highly-responsive cell-based assay utilizing non-coding RNAs
- Rapid and cost-effective method for toxicity testing using microdevices

Non-coding RNAs are RNA molecules that are not translated into proteins. Recent transcriptomic and bioinformatic studies indicate that the thousands of non-coding RNAs exist, and newly identified non-coding RNAs dynamically regulate the gene expression in mammalian cells.

We hypothesized that non-coding RNAs highly respond to environmental stresses, such as ecotoxicological substrates. We have developed a highly susceptible cells that died by environmental stresses faster than normal cells. This technique is capable of rapid and sensitive method for toxicity testing.

We focus on the human iPS cells that can differentiate into various cells and tissues. In the future, we will assess the ecotoxicity of environmental samples to each human tissue using human iPS cells. Moreover, we will developed a rapid and cost-effective devices for ecotoxicity testing.
Detection of microorganisms using optical disk and image recognition technologies

- Early detection without culturing microorganisms
- Rapid scanning and low cost system using optical disk
- Identification of the organisms by image recognition and DNA probe hybridization techniques

**Research target**

Detection and identification of pathogenic microorganisms such as *Escherichia coli* within several hours without cultivation by combined use of our original technologies including optical disk techniques and image recognition based on HLAC (Higher-order Local Auto Correlation).

**Research content**

We measure the light intensity from an optical disk when microorganisms are attached and rebuild an image of the microorganisms by scanning many grooves and aligning the results properly. We roughly identify microorganisms from the cell shapes by pattern recognition techniques based on HLAC. We also use DNA probe hybridization technique for more accurate identification of microorganisms based on the fluorescently-labeled probe.

**Comparison with other techniques (Advantages are shown in red.)**

<table>
<thead>
<tr>
<th></th>
<th>Optical Microscope</th>
<th>Optical Disk</th>
<th>Cultivation (e.g., <em>E. coli</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recognition of the attachment</td>
<td>Just after the measurement</td>
<td></td>
<td>Cultivation time: &gt; 1 day</td>
</tr>
<tr>
<td>Identification</td>
<td>Simple test within several hrs</td>
<td></td>
<td># 1-3 days for simple test; # 5 days for definitive test</td>
</tr>
<tr>
<td>Scanning time (Area: 100 cm²)</td>
<td>22 hrs (2 s /img. @40x¹)</td>
<td>0.2 hrs (@DVD 6x)</td>
<td></td>
</tr>
<tr>
<td>Expertise</td>
<td>High</td>
<td>Low</td>
<td>Extremely high</td>
</tr>
<tr>
<td>Apparatus cost</td>
<td>$10k - $50k</td>
<td>$50k (initial stage) $100 (as of DVD)</td>
<td>$30k - $50k</td>
</tr>
<tr>
<td>Inspection cost</td>
<td>$1 - $2 (Glass sub.)</td>
<td>$1 - $2 (Polycarbonate sub.)</td>
<td>$20 - $50²</td>
</tr>
</tbody>
</table>

S: in US dollars, ¹: an assumption with no automatic-scanning stage, ²: depending on with or w/o the employment costs
Detection of Microorganisms in Water Using Optical Disk and Image Recognition Technologies
Measurement Setup

- Fluorescence Detector
- Filter
- Disk Substrate
- Optical Pickup
- Microorganisms

Measurement Setup

- Laser
- Optical disk based technology
- Reflected light
- Fluorescence

Drinking Water & Agricultural Water

To Ensure Security & Safety of Your Life

- Prompt action when identified
- Identify microorganisms by image recognition and/or fluorescence detection

Identification of microorganisms by reflected light and fluorescence detection

Light Intensity
- Reflected light
- Fluorescence

Scanned Length (or Time)
- Micro-organism: 100 nm - 10 μm

Microbial detection without cultivation

To Ensure Security & Safety of Your Life
Shape analysis by pattern recognition

Focused laser spot

Imaging by optical disk signals

Reflectance

Fluorescence

Land & Groove Substrate

Bacterium-A Dusts Bacterium-B

Multivariate analysis

Vectors are projected to feature space and mathematically categorized.

Feature ext.

Pattern space

HLAC (Higher-order Local AutoCorrelation)

N-dimensional vector

N masks

Identification

Classification

Feature space

AIST

National Institute of Advanced Industrial Science and Technology
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<tr>
<td>Scanning time (Area: 100 cm²)</td>
<td>22 hrs (2 s /img. @40x†)</td>
<td>0.2 hrs (@DVD 6X)</td>
<td>TBC</td>
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Optical disk sensor (prototype)

Optical disk drive
(Measurement)

Signal processing unit
(Analysis)
Smart Sensor Network for Environmental Management

- Cloud-based water quality monitoring & controlling sensor network system
- Both simple measurement using SNSs and large-scale analysis using cloud are possible
- Also applicable to power consumption management and home security system

The water management system is a large-scale distributed system which monitors and controls water resource, and its key to high reliability and low cost is utilization of the cloud computing. We have developed several hardware and software combining various sensors and the cloud systems, and have realized both simple and easy monitoring using social network services such as Twitter® and large-scale information accumulation and analysis using cloud services such as Google App Engine®.
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Membrane bioreactor (MBR)

Wastewater treatment process;
Combination of membrane process and activated sludge (AS)

**Conventional**

**Degradation**

- Waste-water
- Organics
- $\text{CO}_2$
- $\text{O}_2$

**Precipitation**

- Supernatant
- Release

**Drawbacks**

- Precipitation requires
  - a large ground area
  - a long time
  - control of AS conditions

**MBR**

**Degradation**

- Waste-water
- Organics
- $\text{CO}_2$
- $\text{O}_2$

**Filtration**

- Filtrate
- Release

**Advantages**

- Small space
- High density microbes degrade organics at higher rates
Current concerns of MBR operation

• Market size is growing  
  ⇒ Becoming more important

![Bar chart showing growth in the global market for MBR from 1990 to 2013.](http://www.cswea.org/events/2011edseminar/)

(Prof. TorOve Leiknes (2011): Key elements and bottlenecks of the membrane bioreactor (MBR) process for advanced wastewater treatment, CSWEA Education Seminar)

http://www.cswea.org/events/2011edseminar/

• MBR requires experienced operators  
  ⇒ Deal with case-by-case problems

**Indicators of reactor condition are required for stable performance**
Objectives of this study

Make criteria for MBR operation not empirically dependent

(1) Under optimum performance
⇒ How microbial community changes?
⇒ How the community relates to performance?

Investigate microbial community changes at high resolution by next-generation sequencing

(2) Identification of microbes requires long analysis time
⇒ Any other indicator?

Focus on supernatant proteins in MBR
Design and operation of pilot scale MBR

Flux
- Volume: 230 L
- Wastewater (in): 115 L/day
- Treated water (out): 115 L/day
- HRT: 2 days

Measurements
- MLSS
- COD
- TOC
- TN
- Amines (NH₃, DMA, TMA)
- DO
- Flow rate, Transmembrane pressure
- Microbial community …etc.

Organic loading change [TOC]
- 1130 mg/L: first 1 week
- 2260 mg/L: following 2 weeks

Organic loading was increased by 2 times during the operation
⇒ Link the reactor performance to microbial community
Next-generation sequencing (NGS) analysis

Illumina MiSeq Data/run
- Total yield: ~ 15 Gb
- Sequence length: 300 b × 2
- Read number: ~ 50 millions
- Sample number: ≈ 200 by using tags

Sequence data analysis
- Sequence join (ea-utils), q30 quality selection (qiime), chimera seq remove (mothur), phylogenetic analysis (qiime)

This method identifies millions of species/run
⇒ 30,000~60,000 reads/sample
Changes of microbial community at Class-level

All sludge samples were analyzed by NGS
⇒ Classified at “Class”-level

Under high organic loading conditions
⇒ Microbial community was drastically changed
⇒ \textit{\textbf{\textcolor{red}{$\gamma$-proteobacteria}}} and \textit{\textbf{\textcolor{blue}{Bacteroidetes}}} became dominant.
Stable isotope probing (SIP)

A powerful tool to identify the function of uncultured microbes

- Cultivation of microbes with stable isotopes
- Nucleic acids extraction & density gradient centrifugation
- High sensitive detection of the labeled microbes

Conventional T-RFLP fingerprinting

Advanced

Combined with deep sequencing
A slight portion (0.001%) of $^{13}$C-labeled RNA was identified phylogenetically
Four species of microbes were accumulated in the heavy fraction
These were involved in degradation of palmitate, (an oil component)

A combination of SIP and deep sequencing enabled the sensitive identification of $^{13}$C-assimilating microbes
Summary of this presentation

1. Microbial community

Under high organic conditions,
- Anaerobes and related species were highly increased
- Coexistence is important

2. Proteins

Under high organic conditions,
- Several proteins were found, and correlated to the conditions
- They can be used as indicators ⇒ Continue to analyze
Summary (MBR)

- Construction and operation of the pilot-scale MBR
- Demonstration of the microbial community dynamics in the MBR with high resolution using next-generation DNA sequencer
- SIP combined with 16S rRNA deep sequencing successfully identifying the key players in the activated sludge

**NEXT FOCUS: MBR and RO system**

1) Stable and efficient control of microbial community by continuously monitored physicochemical parameters.
2) Biofouling mechanism of membrane by physicochemical and microbial analyses.
Nanocomposite for Cleaning Environmental Pollutants

- Novel hybrid nanostructures of carbon nanosheets (1 to tens graphene layers) and metal / metal oxide nanoparticles achieving synergy of adsorption and catalysis

Research target

Aiming at efficient removal of trace chemicals (POPs, PPCPs, etc.) which have potential impacts on human beings and ecosystem, we are developing technology for nanostructured composition of graphene (G) and metal/metal oxides. Nanocomposite from titania and G demonstrates a high efficient adsorption concentration-induced photocatalysis.

Research content

By combining intercalation and hydrothermal methods, a synthesis technique which utilizes carbon nanosheets (CNS) as a template for 2D deposition of 1D titanate nanotube (TNT) or nanorod (TNR) was developed. Through adsorption concentration of the substrate CNS, photoactivity of the composite was promoted by 5 to 6 times as compared to the pure titania.
Water purification by solar power

**Research target**

- Drinking water purification in developing country
- Research of photo catalytic system adjusted to the local area
- International collaboration

E. Coli treatment results. $1 \times 10^6$ cfu/mL of E. coli was successfully disinfected within 8 hours. (1L)

**Research content**

-タイ・チェンライ県におけるサンプリング調査
  - 民族と生活レベルに大きな差
- タイ・少数民族集落(チェンライ県)の飲料水並びに生活用水中の大腸菌並びに一般細菌群。WHO基準から見ると飲用不可レベル。
Vietnam
VAST
JSPS Bilateral Programs
MBR, Photocatalyst

Thailand
TISTR, NSTDA (NANOTEC)
Field Experiment for Sterilization of Drinking Water

China
Research exchange
Tsinghua Univ., Sichuan Univ.
Nano-materials for Water Treatment

Indonesia (national institutes, companies and universities)
Singapore
India