Novel function of polyaniline for biological environments: Cultivation of paramecium in the presence of polyaniline

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Abstract. The reduced form of polyaniline (PANI, emeraldine base) functions as water purification to extend the lives of paramecia. The emeraldine base can absorb discharged waste from the planktons such as nitrogenous compounds and salts. This is a new function of \(\pi\)-conjugated polymers for micro-organisms.

1. Introduction
Leuwenhoek (the Netherlands) firstly observed paramecium under optical microscopy [1]. Movement of the paramecium in water is by its cilia. In the closed environment, such as water in a small beaker, paramecium cannot live for long. Increase of population of paramecia in a beaker is observable at first, however, at some point the population suddenly decreases because of discharged wastes, such as nitrogenous compounds and salts. The water waste from paramecia decimates the paramecium population itself in such the restricted circumstance. Planktons have no ornithine cycle, so they cannot convert ammonia to nontoxic urea. The discharged ammonia pollutes their living environment. Fortunately, in the natural realm, the circulation system detoxifies unwanted materials and provides good conditions for living organisms.

Polyaniline (PANI) is one of the most promising conducting polymers because it possesses relatively high electrical conductivity, corrosion resistance [2], and antistatic functions [3]. Recently, radioactive-atom adsorption function of PANI for air cleaning system has been proposed [4]. The emeraldine base (EB) of the PANI can absorb electron acceptors (acids) by the electron donor-acceptor (dopant) interaction. This research explores a new function of PANI for collection of pollutants.

In this report, cultivation of paramecia in the presence of PANI emeraldine base (PANI-EB, half-doped form) is examined.

2. Paramecium
Three kinds of paramecia, G3 paramecium caudatum, Sol5/white paramecium bursaria, and Sol5/green paramecium bursaria were employed in this study. A small amount of chlorella is placed in the water for food for the paramecia.

2. Synthesis
2.1. Polyaniline
A solution of aniline (1 g) and sulfuric acid (1 g) in water (200 mL) was stirred for 1 h. The solution was cooled by ice. Then, ammonium peroxodisulfate (1.5 g) was slowly added to the solution. After 24 h, the solution was filtered. The crude PANI cake on the funnel was collected, and washed with a large volume of water (1000 mL). The solution was filtered again. The solid was collected and washed with a large volume of methanol (200 mL). After a further filtration, the solid was washed with a large volume of ammonia/water. The colour of PANI changed from dark green to dark blue upon the...
treatment with the ammonia solution (Scheme 1). The product was collected after filtration, and dried in vacuum to yield PANI-EB. PANI before treatment with ammonia was also obtained as PANI emeraldine salt (PANI-ES, doped form).

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\text{NH}_4^+ + \text{H}_2\text{O} \rightarrow \text{APS} = \text{ammonium persulfate}
\]

**Scheme 1.** Synthesis of polyaniline emeraldine salt (PANI-ES) and emeraldine base (PANI-EB). APS = ammonium persulfate.

2.2. Poly(pyridine-vinylene-azobenzen), P(Py-Vy-Az)

The copolymerisation was carried out by the Migita-Kosugi-Stille coupling polycondensation followed by Mizoroki-Heck reaction with the one-pot synthesis method. Synthetic route for poly(pyridine-vinylene-azobenzen) (P(Py-Vy-Az)) is shown in Scheme 2. \(p\), \(p\)'-Dibromoazobenzene (AzBr\(_2\)) was prepared from \(p\)-bromonitrobenzene by using MnO\(_2\), according to the literatures [5,6]. A solution of 2,6-dibromopyridine (0.3 g, 1.28 mmol), \([\text{tris(dibenzylideneacetone)dipalladium(0)}]\) (Pd(dba)\(_3\), 22 mg, 0.24 mmol), tris(\(o\)-furyl)phosphine (P(fulyl)\(_3\), 24 mg, 0.24 mmol), tributyl(vinyl)tin (0.8 g, 2.5 mmol) in tetrahydrofuran (THF, 1 mL) was refluxed for 24 h at 60 °C. Another solution of \(p\), \(p\)'-dibromoazobenzene (0.43g, 1.28 mmol), palladium diacetate (II) [Pd(OAC)\(_2\)] (56 mg, 0.25 mmol), tris(\(o\)-tolyl)phosphine (P(o-tol)\(_3\), 0.4 g, 1.3 mmol) in \(N\),\(N\)-dimethylformamide (DMF, 1 mL) was added and stirred for 3 days at 60 °C. Then, the reaction mixture was poured in a large volume of methanol (ca. 200 mL), and HCl was added to the solution to remove the catalysts. After filtration, the product was washed with methanol containing ammonia/H\(_2\)O solution. The polymer was collected by filtration, and dried in vacuum to yield 0.52 g of dark red powder.

**Scheme 2.** Synthetic route for poly(pyridine-vinylene-azobenzen), P(Py-Vy-Az). Pd(dba)\(_3\) = tris(dibenzylideneacetone)dipalladium(0), P(fulyl)\(_3\) = tris(\(o\)-furyl)phosphine, THF = tetrahydrofuran, Pd(OAC)\(_2\) = palladium diacetate (II), P(o-tol)\(_3\) = tris(\(o\)-tolyl)phosphine, Bu\(_3\)Sn = tetrabutyl tin, DMF = \(N\),\(N\)-dimethylformamide.

3. Cultivation of paramecia

Paramecia in water were added to the water containing the PANI-EB or P(Py-Vy-Az). The PANI-EB and P(Py-Vy-Az) were insoluble in the water, so the fine particles of the polymers were dispersed.

The paramecia moved in the water in the presence of the polymers (Figure 1), although the paramecia could not live in the water containing the PANI-ES (full-doped form) because of strong acidity (pH < 4.3).
4. Observations
Oxidation-reduction (redox) potentials of water containing paramecia were measured as references. The oxidation-reduction potential (ORP) values were to be 281–348 mV (Table 1).

Figure 1. Paramecia with polyaniline emeraldine base (PANI-EB) in the water (left). A magnification image (right).

Table 1. ORP, pH measurement results.

| Paramecium | ORP\(^3\) (mV) | pH (initial) | pH (final) |
|------------|----------------|--------------|------------|
| G3 P. caudatum\(^1\) | 312 | 7.6 | 7.6 |
| Sol5/white P. bursaria\(^1,2\) | 281 | 7.5 | 7.6 | 8.2 |
| Sol5/green P. bursaria\(^1\) | 348 | 7.7 | 7.1 | 7.9 |

\(^1\)P = paramecium.
\(^2\)Sol5/white paramecium bursaria lived in the water containing P(Py-Vy-Az) for 10 days.
\(^3\)ORP = oxidation-reduction potential.

Figure 2. Differential interference optical microscopy image of a paramecium and PANI-EB in the water (left). Survival period of the paramecia in the presence of PANI-EB (right).

A differential interference optical microscopy image confirmed a figure of the paramecium (Figure 2, left). The paramecia in the flask could live for a long period in the water containing the PANI-EB (Figure 2, right). The PANI-EB is in a half-doped state. Therefore, the discharged wastes from the paramecia were neutralized by the PANI-ES. However, a paramecia in the water containing
P(Py-Vy-Az) lived for only 10 days. P(Py-Vy-Az) has no acid because it is the undoped form. The solution containing P(Py-Vy-Az) was not neutralized and the water was polluted by the material from the paramecia. Discharged wastes from paramecia in the water were absorbed by the PANI-EB. The water purification function of the PANI-EB provides a good environment for long paramecium life. The pH value of the solutions are increased except for G3 P. caudatum, as shown in Table 1, indicating base substances such as ammonia from paramecia could be neutralized by the PANI-EB. Therefore, the PANI-EB provides a good environment for the paramecia.

5. Conclusions
At present, components of discharged wastes cannot be clearly identified because living organisms discharge many kinds of compounds. However, the longevity of the paramecium in the presence of the PANI-EB implies a water cleaning function of the PANI. Mechanical adsorption of discharged wastes by micro-pores of the PANI surface is also considerable. This is the first example of the application of polyanilines to the biological environment, and thus the possibility of π-conjugated polymer utility was further expanded.

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References
[1] Palm L C 1982 Antoni van Leeuwenhoek, 1632–1723: Studies on the Life and Work of the Delft Scientist Commemorating the 350th Anniversary of His Birthday (Amsterdam: Rodopi BV)
[2] Chen X J, Luo C, Zhang Z H and Zhao M 2012 Anti-Corrosion Methods and Materials, 59 291
[3] Skotheim T J, Elsenbaumer R L and Reynolds J R 1998 Handbook of Conducting Polymers, 2nd Ed., 1097, (New York: Marcel Dekker)
[4] Goto H 2011 Polymers 3 875
[5] Wheeler O H, Gonzalez D 1964 Tetrahedron 20 189
[6] Sandler S R, Karo W 1971 Organic Functional Group Preparations, Vol II (New York and London: Academic Press) p321