Enhancement of the Direct Electron Transfer-type Bioelectrocatalysis of Bilirubin Oxidase at the Interface between Carbon Particles

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ABSTRACT

The simulation model of the direct electron transfer (DET)-type bioelectrocatalysis at a porous electrode indicates that the catalytic current is proportional to the real surface area (A), when A is small. When A is large, the catalytic current is saturated at a limited value controlled by the mass transfer in the porous structure. In this study, the bioelectrocatalytic currents at porous electrodes are analyzed based on their charging currents. Comparisons among the carbon composites constructed with Vulcan and exfoliated graphite (JSP) show that the interface between the Vulcan and JSP particles is more suitable for the DET-type bioelectrocatalysis of bilirubin oxidase (BOD) than that between the particles of the same type. In particular, the most suitable properties were achieved at a Vulcan : JSP ratio of 1 : 1 in the composite electrode. In these composites, the multijunction point between the BOD molecule and electrode seems to result in a higher DET-type bioelectrocatalytic activity.

1. Introduction

A bioelectrocatalytic reaction is the coupled reaction of the redox enzymatic reaction and the electrode reaction. When there is direct electron transfer between the enzyme and electrode, the coupled reaction is referred to as a direct electron transfer (DET)-type bioelectrocatalytic reaction.1–12 Bilirubin oxidase (BOD) is one of the multicopper oxidases that can be used for the four-electron reduction of oxygen to water through DET-type bioelectrocatalysis.13,14 BOD is widely employed as a biocathode catalyst in biofuel cells15,16 because of its relatively high activity and relatively positive potential17 in DET-type bioelectrocatalysis.

The electron transfer between the active redox center in the enzyme and the electrode is due to the tunneling effect.8,15 Therefore, the distance of the electron transfer pathway and the orientation of the adsorbed enzyme at the electrode surface are considerably important in DET-type bioelectrocatalysis. BOD has two redox centers, a type 1 copper (T1) site and a type 2 copper (T2) site, in its structure.16–18 The T1 site is regarded as the electrode active redox center of BOD.19 The surface of BOD in the vicinity of the T1 site is positively charged for the BOD derived from Myrothecium verrucaria.20,21 Therefore, the modification of the electrode surface with negatively charged aromatic compounds effectively improves the DET-type bioelectrocatalysis of BOD from Myrothecium verrucaria.16,20,22–27 The different orientations of the adsorbed enzyme cause a distribution of the distance between the redox center and electrode. The distribution of the distance distorts the shape of the voltammogram of the DET-type bioelectrocatalytic process.5,28

Porous electrodes are effective scaffolds for DET-type bioelectrocatalysis.7–31 Previously, the DET-type bioelectrocatalysis of BOD has been improved on porous carbons,31 porous metals,32 accumulated metal nanoparticles,3,23 carbon nanotubes,34,35 and accumulated graphene-modified electrodes.36,37 According to the modeling of the DET-type bioelectrocatalysis of a spherical enzyme in a pore,9,23 the nanometer-sized pores at the electrode surface are important for the reaction. In the nanometer-sized pores, the enzyme makes multiple contacts with the electrode surface. The multiple contacts improve the probability of the enzyme has the effective orientation. Therefore, an electrode with nanometric pores on its surface is more advantageous than a planar electrode for the DET-type bioelectrocatalysis with an enzyme. The improvement in the DET-type bioelectrocatalysis on a porous structure has been explained by the curvature effect at the electrode surface.9

The optimization of the enzyme orientation and the porous nanostructure at the electrode surface is important to improve the performance of the bioelectrode. However, it is difficult to separate the contributions of the surface property and the curvature effect at the electrode surface. In particular, analytical approaches for evaluating the DET-type bioelectrocatalysis at porous electrodes have been limited because the porous structure can affect the mass transfer of the substrate. As heterogeneous porous structures present many difficulties in determining the real surface area and identifying the surface composition, the comparisons among the electrode materials are frequently carried out on the basis of the amount of the porous material18,39 rather than on the electrochemically effective surface area.

The determination of the electrochemically active surface area of a porous electrode is challenging.40 The non-Faradaic current, which is proportional to the electrochemically active surface area, seems to be the most convenient parameter for evaluating it. Although it is assumed that the double layer capacities of the electrodes are equal, the charging current is considered to be a good indicator of the DET-type bioelectrocatalysis on a porous electrode. In our previous work,41 the DET-type bioelectrocatalytic reactions of BOD at the surface of various carbon particles, such as Ketjen Black (KB), Vulcan, and high-purity exfoliated graphite (JSP), were compared on the basis of the charging current. Moreover, the study showed that the interparticle interfaces between the primary particles of KB and Vulcan (KB/JSP and Vulcan/Vulcan) are effective for the DET-type bioelectrocatalysis with BOD. Additionally, it was found that the hollow interior of the KB particle is not useful for DET-type bioelectrocatalysis. On the other hand, JSP, which is the largest particle among the studied carbon particles, had the most suitable surface for the DET-type bioelectrocatalysis of BOD.
In this study, the effect of the porous structure of the electrode on the mass transfer in the DET-type bioelectrocatalytic reaction was simulated by the finite element method. To investigate the interface between the carbon particles, composite electrodes were constructed using Vulcan and JSP, and the surfaces of the composite electrodes were visualized by scanning electron microscopy. The DET-type bioelectrocatalytic currents of BOD in these composites are compared on the basis of the charging currents. Finally, the orientations of BOD at these composites are discussed according to the random orientation model.

2. Model

Porous electrodes constructed with hexagonally close-packed spheres were considered for modeling. The spheres were assumed to be conductors, and the contact resistance was ignored. The space between the spheres was filled with an electrolyte solution. The substrate was able to diffuse freely in the electrolyte solution. According to the sizes of the carbon particles used in experimental studies, the diameter of the sphere \( d_b \) was set to 40 nm or 10 \( \mu \)m. The thicknesses of the porous layer and diffusion layer in the solution phase are denoted as \( l_p \) and \( l_d \) respectively. The geometry of the model is shown in Fig. 1A. The DET-type bioelectrocatalytic reaction occurs at the surface of the spheres. To express the substrate consumption, the enzymatic reaction rate per unit area was calculated using the Michaelis–Menten equation:

\[
y = \frac{k_c \Gamma_s c_s}{K_M + c_s},
\]

where \( c_s \) is the substrate concentration, \( K_M \) is the Michaelis constant, \( k_c \) is the catalytic constant, and \( \Gamma_s \) is the adsorbed amount of the enzyme per unit area of the real surface. Assuming the limiting current conditions, the kinetics of the electrode reactions were ignored in this model. The bulk concentration of the substrate outside the diffusion layer was kept constant at \( c_{sb} \).

The real surface area of the electrode \( (A) \) was calculated as the summation of the surface areas of the spheres. The ratio of the real surface area to the projective surface area \( (A/A_0) \) is employed as the index of the porosity of the electrode. In this model, \( A/A_0 \) of 3.63 corresponds to a monolayer of close-packed spheres. The steady-state solution of the model was obtained using a commercially available finite element method package (COMSOL Multiphysics 5.5, COMSOL, Inc., USA). The steady-state current \( (j_{ss}) \) of the system was calculated by the following integration for the cross-section of the system (s):

\[
j_{ss} = -\int_{s} n_{s} F D_{s} \frac{dc_{s}}{ds},
\]

where \( n_{s}, F, \) and \( D_{s} \) are the electron number of the substrate, Faraday constant, and diffusion coefficient of the substrate, respectively.

3. Experimental Section

3.1 Materials

BOD (EC 1.3.3.5) from Myrothecium verrucaria was purchased from Amano Enzyme Inc. (Japan), and used without further purification. Carbon materials such as Vulcan (primary particle size: 30–40 nm), Brunauer-Emmett-Teller (BET) surface area: 230 \( \text{m}^2 \text{g}^{-1} \) and JSP (primary particle size: 10 \( \mu \)m, BET surface area: 11 \( \text{m}^2 \text{g}^{-1} \) ) were purchased from Cabot Corporation (USA) and Nippon Graphite Co., Ltd. (Japan), respectively. Poly(1,1,2,2-tetrafluoroethylene) fine powder 6-J (PTFE) was purchased from DuPont-Mitsui Fluorochemicals Co., Ltd. (Japan). Unless otherwise specified, all other chemicals were of analytical grade and purchased from Wako Pure Chemical Industries, Ltd. (Japan). All solutions were prepared with ion-exchanged water.

3.2 Electrode preparation

Glassy carbon (GC) electrodes with a diameter of 3.0 \( \text{mm} \) (BAS, Japan) were polished with 0.05 \( \mu \)m alumina slurry and then cleaned by sonication and washing with ion-exchanged water. Each carbon material (40 mg) was mixed with PTFE (10 mg) as a binder and 3.5 mL (L = \( \text{dm}^3 \)) of 2-propanol, and the mixture was homogenized with an ultrasonic homogenizer for 10 min. Thereafter, 6.5 mL of ion-exchanged water was added to the slurry, and the mixture was homogenized for 5 min with an ultrasonic homogenizer.

An appropriate amount of the carbon material mixture was cast on the surface of the polished GC electrode and dried at room temperature to evaporate the solvent. In the preparation of the carbon composite electrode, the homogenized slurries of JSP and Vulcan were mixed at the volume ratios of 1 : 9, 5 : 5, and 9 : 1 and homogenized for 1 min with sonication before casting. The corresponding composites are referred to as 1J9V, 5J5V, and 9J1V, respectively.

BOD (0.05 g) was dissolved in 250 \( \mu \)L of a 0.1 M \( (M = \text{moldm}^{-3}) \) phosphate buffer (pH 7.0), and 10 \( \mu \)L of this BOD solution was spread on the carbon-material-modified GC electrodes. The electrodes were then allowed to stand in a water-saturated atmosphere for 1 h at 4 \( ^\circ \)C. The enzyme-adsorbed carbon-material-modified electrodes were washed with a phosphate buffer solution (pH 7.0) before electrochemical measurements.

3.3 Electrochemical measurements

All electrochemical measurements were conducted using an electrochemical analyzer (CompactStat, Ivium Technologies, Netherlands). Steady-state voltammetric measurements were carried out with rotating disk electrodes (RDEs) (RDE-1, BAS, Japan) at a rotating speed \( (\omega) \) of 4000 rpm and scan rate \( (\nu) \) of 10 mV s\(^{-1}\) in an O\(_2\)-saturated 0.1 M phosphate buffer solution (pH 7.0). A platinum wire and Ag/AgCl/1 sat. KCl electrodes were used as the counter and reference electrodes, respectively. All potentials in this work are reported with respect to the reference electrode.

3.4 Scanning electron microscopy (SEM)

The surfaces of the carbon-material-modified electrodes were observed with a field emission scanning electron microscope (TM4000, Hitachi High-Technologies Co., Japan) at an acceleration voltage of 15.0 kV.
4. Results and Discussion

4.1 Mass transfer and Faradaic processes at porous electrodes

Figure 1B shows the bioelectrocatalytic current at the porous electrode when \( I_d = 10 \text{ µm} \) and \( K_{ut} = 0.2 \text{ mM}^{-1}. \) In this study, the current density \( (j) \) is defined as the current per projective unit area of the electrode. According to the Levich equation for oxygen in an aqueous solution (bulk concentration \( (c_o) \) of 1.2 mM,20 electron number \( (n) \) of four, diffusion coefficient \( (D) \) of \( 2.1 \times 10^{-9} \text{ m}^2 \text{s}^{-1}, \) and kinematic viscosity \( (\nu) \) of \( 0.89 \times 10^{-6} \text{ m}^2 \text{s}^{-1}, \) the value of \( I_d \) corresponds to that of the electrode at a rotating speed of 4000 rpm. The filled triangles and filled diamonds in Fig. 1B show the results for the JSP model \( (d_o = 10 \text{ µm}) \) and the Vulcan model \( (d_o = 40 \text{ nm}) \) with \( k_d \Gamma = 5 \times 10^{-6} \text{ mol s}^{-1} \text{ m}^{-2}, \) respectively. The increment in the surface area (that is, the widening of the porous layer) increased linearly with the steady-state current. Additionally, the steady-state currents in the low \( A/d_o \) region were independent of the size of the particles. The open diamonds in Fig. 1B indicate the steady-state current for the Vulcan model calculated at \( k_d \Gamma = 2 \times 10^{-6} \text{ mol s}^{-1} \text{ m}^{-2}. \) The slope of this region is expressed by \( nFk \Gamma. \) Therefore, it is important to compare the bioelectrocatalytic activities of enzymes adsorbed at different porous electrodes according to the real surface area of the porous electrode.41

On the other hand, the maximum values of the steady-state currents are 7.5 and 1.2 mA cm\(^{-2}, \) respectively, for the Vulcan and JSP model at \( k_d \Gamma = 5 \times 10^{-6} \text{ mol s}^{-1} \text{ m}^{-2}. \) The difference in the maximum current suggests that the maximum value of the current is limited by the diffusion of species in the pores. Unfortunately, the maximum currents also depend on the value of \( k_d \Gamma \) (shown in Supporting information (Fig. S1)). It is not possible to compare the surface properties based on the maximum current because the value is strongly affected by the porous structure. A comparison of the porous electrode is possible only when the porosity of the electrode is sufficiently low. The simulation shows that the increase in the surface area per unit volume of the porous electrode is important for amplifying the substrate-transfer-limiting current.

4.2 Orientation of BOD at various carbon composites

Figure 2 shows the rotating-disk cyclic voltammograms (RDCVs) of the \( \text{O}_2 \) reduction through the DET-type bioelectrocatalysis of BOD adsorbed on electrodes modified with a large amount of various carbon components, (solid lines) 5J5V, (long-dashed lines) 1J9V, (short dashed lines) JSP, (dot-broken lines) Vulcan, and (dotted line) 9J1V. Clear sigmoidal voltammograms were obtained except for the case of 9J1V. The voltammograms suggest that BOD worked well as a DET-type bioelectrocatalyst in the four-electron reduction of \( \text{O}_2 \) at the surface of the carbon materials.

When a large amount of the carbon material was present at the electrode surface, the largest current was recorded with the Vulcan-modified electrode. The maximum value is approximately \(-3.5 \text{ mA cm}^{-2}. \) On the other hand, the catalytic currents for the electrodes containing JSP were limited to approximately \(-1.5 \text{ mA cm}^{-2}. \) According to the simulation model, the catalytic current at the porous electrode will be limited by the mass transfer of the substrate. Therefore, the large particles of JSP in the carbon composites will limit the mass transfer of the substrate in the porous carbon materials. Additionally, the substrate-transfer-limited current is insensitive to the ratio of JSP and Vulcan in the carbon composites. The catalytic and charging currents for the 9J1V modified electrode are quite small. Since the charging current is independent in the casted amount of 9J1V at the electrode surface (Fig. S2), the number of conductive paths in this composite is quite small.

Figure 3 shows the SEM image of the surface of each carbon composite. At the resolution of the images, the primary particles of Vulcan are not identified. The surface of the 1J9V layer is mainly covered with Vulcan. In the case of 5J5V, the primary particles of JSP coated with Vulcan are separated from each other. On the other hand, in the case of 9J1V, the bare surface of JSP is exposed. Therefore, there is no attractive interaction between JSP particles and Vulcan particles.

To investigate the effect of the composite surface on the DET-type bioelectrocatalysis of BOD, the orientation of the adsorbed BOD was analyzed based on the random orientation model.20 When small amounts of the carbon materials are present at the electrode surface, the bioelectrocatalytic current is predominantly limited by the enzymatic reaction of BOD. Figure 4 shows the RDCVs for small amounts of carbon composites with a maximum current of approximately \(-0.3 \text{ mA cm}^{-2}. \) Because the bioelectrocatalytic currents are smaller than the substrate-limited value, the currents are mainly controlled by the enzymatic reaction. In these voltammograms, the onset potential of the bioelectrocatalytic oxygen reduction appeared at \( 0.5 \text{ V}. \) These voltammograms do not have a clear sigmoidal shape. The bioelectrocatalytic oxygen reduction current depended linearly on the applied potential in the potential region more negative than \(-0.4 \text{ V}. \) The slope of the voltammograms is referred to as the residual slope.6,15,20 The existence of the residual slope on the voltammogram indicates the randomly oriented enzyme molecules at the electrode surface.

Although the catalytic currents were lower than the diffusion-limited currents, the contribution of mass transfer to the catalytic
current was still large. To eliminate the effect of mass transfer on the catalytic current, a serially connected resistances model is considered here. Considering bioelectrocatalysis at the porous RDE, the $j_\text{value}$ can be given by the following Koutecký-Levich-type equation combined with the mass transfer in the porous structure:

$$
\frac{1}{j} = \frac{1}{j_\text{a}} + \frac{1}{j_\text{b}} + \frac{1}{j_\text{p}},
$$

(3)

where $j_\text{a}$, $j_\text{b}$, and $j_\text{p}$ are, respectively, the limiting current densities controlled by the mass transfer of $O_2$ in the bulk solution at the RDE, the enzymatic reaction kinetics, and the mass transfer in the porous structure. In the case of the four-electron reduction of oxygen, the $j_\text{a}$ value in an $O_2$-saturated aqueous solution can be estimated by the Levich equation to be $8.4 \text{mA cm}^{-2}$ at $4000 \text{rpm}$ and $25 \degree \text{C}$. The value of $j_\text{b}$ is given by the random orientation model,20 as follows:

$$
j_\text{b} = \frac{j_{\text{c, max}}}{\beta \Delta d (1 + \exp(\Phi))} \ln \left( \frac{j_{\text{c, max}}}{k_c} \left( 1 + \frac{\exp(\Phi)}{\exp(\alpha \Phi)} \right) \right),
$$

(4)

where

$$
\Phi = \frac{n_c F}{RT} (E - E^\circ),
$$

(5)

$$
j_{\text{c, max}} = -n_c F k_c \Gamma',
$$

(6)

$\beta$ is the coefficient of the long-range electron transfer, $\Delta d$ is the difference in the distance between the closest and farthest approaches of the redox center of an enzyme that electrochemically communicates with an electrode, $n_c$ is the number of electrons in the rate-determining step of the interfacial electron transfer (= 1 for the T1 site in BOD), $R$ is the gas constant, $T$ is the absolute temperature, $E^\circ$ is the formal potential of the redox center of the enzyme, $k_c$ is the standard rate constant at the distance of the closest approach in the best orientation of the enzyme, and $\alpha$ is the transfer coefficient.

As mentioned above, the theoretical estimation of the value of $j_\text{p}$ is difficult. In this work, the value of $(j_\text{a}^{-1} + j_\text{b}^{-1})^{-1}$ was estimated from the experimentally recorded maximum current when a sufficient amount of the carbon material was present at the electrode surface ($\sim 3.5 \text{mA cm}^{-2}$ for Vulcan and $\sim 1.5 \text{mA cm}^{-2}$ for JSP-containing composites). Using $j_{\text{c, max}}$, $\beta \Delta d$, $k_{\text{c, max}}/k_c$, and $\alpha$ as adjustable parameters, Eq. (3) was fitted to the voltammograms using non-linear regression analysis with Gnuplot. In this fitting, the employed value of $E^\circ$ is $0.46 \text{V}$.44 The parameters corresponding to the best fits to the voltammograms shown in Fig. 4 are listed in Table 1.

The regression analysis for the 9J1V-modified electrode resulted in a large error because the potential-independent current limited by the enzymatic reaction was not observed in the voltammogram.

To compare the carbon materials, an appropriate normalization of the electrode surface area is required. According to the comparison of the BET surface area and charging current, there is no significant difference between the double layer capacitances of Vulcan and JSP (shown in Fig. S2). Here, we employ the charging current as an indicator of the real surface area of the electrode surface. In this analysis, the charging current ($j_\text{b}$) was estimated from the voltammogram as the difference between the anodic and cathodic currents of RDCV at 0.55 V.

According to Eq. (4), the value of $j_{\text{c, max}}/j_b$ is proportional to $k_c \Gamma'$. Vulcan and JSP have almost the same values of $j_{\text{c, max}}/j_b$. On the other hand, 5J5V has the largest value of $j_{\text{c, max}}/j_b$ among the inspected composites (Table 1). This result shows that an appropriate mixing ratio of Vulcan and JSP creates a more suitable scaffold for the bioelectrocatalysis of BOD.

When the surface of the carbon composite is saturated with BOD, the value of $\Gamma'$ is constant for each composite. Under this

### Table 1. Best-fitted parameters to the experimentally recorded voltammograms.

| Parameter            | Vulcan          | JSP             | 19J9V | 5J5V     | 9J1V  |
|----------------------|-----------------|-----------------|-------|----------|-------|
| $j_{\text{c, max}}$/mA cm$^{-2}$ | $-0.359 \pm 0.002$ | $-0.381 \pm 0.002$ | $-0.263 \pm 0.003$ | $-0.476 \pm 0.005$ | $0 \pm 70$ |
| $\beta \Delta d$    | 9 $\pm$ 1       | 10 $\pm$ 1      | 11 $\pm$ 1 | 16 $\pm$ 2 | 0 $\pm 10^4$ |
| $k_{\text{c, max}}$/k$_c$ | 10 $\pm$ 4     | 17 $\pm$ 6       | 18 $\pm$ 6 | 24 $\pm$ 8 | 30 $\pm 6$ |
| $\alpha$            | 0.50 $\pm$ 0.05 | 0.51 $\pm$ 0.05  | 0.50 $\pm$ 0.04 | 0.76 $\pm$ 0.07 | 0.56 $\pm 0.07$ |
| $j_b$/mA cm$^{-2}$  | 0.0048          | 0.0046          | 0.0076 | 0.0018   | 0.0039 |
| $j_{\text{c, max}}$/j$_b$ | 74.3 $\pm$ 0.4 | 82.4 $\pm$ 0.4 | 22.1 $\pm$ 0.3 | 297 $\pm$ 3 | — |
| $k_{\text{c, max}}$/j$_b$  | 700 $\pm$ 300   | 1400 $\pm$ 500  | 400 $\pm$ 100 | 7000 $\pm$ 2000 | — |

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**Figure 4.** Oxygen reduction RDCVs recorded with BOD adsorbed on electrodes modified with (A) 8 µg Vulcan, (B) 80 µg JSP, (C) 16 µg 19J9V, (D) 8 µg 5J5V, and (E) 16 µg 9J1V. The circles represent the best fit of the data to Eq. (3). The data for JSP and Vulcan were reproduced from Ref. 41 with permission from The Electrochemical Society of Japan.
assumption, the value of \(k_0j_{\text{c,max}}/(k_cj_b)\) is proportional to \(k_0\). The estimated value for 5V5J is very large compared to those of other composites (Table 1). However, the results seem to be affected by the difference in the effective amount of BOD for each carbon composite. The surface structure constructed at 55V provides multipoint contact between BOD and the electrode. Therefore, the effective amount of BOD at 55V becomes larger than that at the other composites.

The value of \(\beta d\Delta d\) at 55V is significantly larger than the values at the other carbon composites. The multipoint contact enables that BOD receives electrons from the electrode at various angles. Therefore, the multipoint contact at the electrode surface increases the value of \(\beta d\Delta d\). The expectation of multipoint contact of BOD at 55V surface is supported by the increase in the effective amount of BOD with the increase in \(\beta d\Delta d\).

4.3 Effect of the amount of carbon composites

The DET-type bioelectrocatalysis, fundamentally, depends on the electrochemically active surface area of the electrode. In this work, the background-subtracted current density recorded at 0 V was defined as the limiting current density \((j_{\text{lim}} = j - j_b)\). Figure 5 shows the \(j_b\) value-dependence of \(j_{\text{lim}}\) for various carbon composites. The value of \(j_{\text{lim}}\) increased with \(j_b\) in the low \(j_b\) region and showed saturation behavior in the high \(j_b\) region at every carbon-material-modified electrode, except for 91V. These experimentally recorded trends agree with the relationship between \(A/A_0\) and \(j_b\) shown in Fig. 1B from the model calculation. Additionally, the saturated values of \(j_{\text{lim}}\) in Fig. 5 agree with those in Fig. 2.

Assuming that the value of \(j_{\text{lim}}\) is proportional to \(j_b\) in the low \(j_b\) region, the slopes of the linear regions are 18 ± 1, 34 ± 3, 24 ± 1, and 104 ± 9 for Vulcan, JSP, 1J9V, and 5J5V, respectively. According to the model calculation, this slope is proportional to \(k_cT_1\). Moreover, the value of \(j_{\text{c,max}}/j_b\) is also proportional to \(k_cT_1\). Both the results shown in Table 1 and Fig. 4 indicate that the surface of 55V is the most effective scaffold for the DET-type bioelectrocatalysis of BOD. In the case of 139V, the expectation of the surface property from the slope in Fig. 4C disagrees with that from \(j_{\text{c,max}}/j_b\). This discrepancy is possibly due to the difference between the experimentally determined \(j_{\text{lim}}\) and \(j_b\) calculated with the model. The model calculation assumes the limiting current conditions for bioelectrocatalysis. However, the experimentally recorded current is still under the effect of the residual slope. Therefore, further analysis of the BOD orientation combined with the mass transfer limitation in the porous electrode is required.

4.4 Effects of the lamination of carbon materials

The above discussion is for homogenized carbon composites. The effect of the lamination of the carbon materials was investigated further. Figure 6 shows the bioelectrocatalytic current for the composite materials with (A) Vulcan overlaid on the JSP layer and (B) JSP overlaid on the Vulcan layer.

When the Vulcan layer is formed on the JSP layer, the value of \(j_{\text{lim}}\) increases with an increase in the casted amount of Vulcan. The saturated value of \(j_{\text{lim}}\) agrees with that of the Vulcan-modified electrode. When a large amount of Vulcan is added, the JSP particles are buried within Vulcan. Therefore, the composite seems to work like a Vulcan electrode when a sufficient amount of Vulcan was cast on the JSP layer.

Interestingly, the increment in \(j_{\text{lim}}\) in the low \(j_b\) region is steeper. The estimated slope in the low \(j_b\) region is 52 ± 4, which is larger than that for Vulcan or JSP. Therefore, Vulcan casted on the JSP layer can be inferred to have led to Vulcan-coated JSP particles, as in the case of 55V, at the electrode surface. An increase in the casted amount of JSP in the Vulcan layer leads to a decrease in the value of \(j_{\text{lim}}\). The decreased value of \(j_{\text{lim}}\) is lower than the value of the saturated current recorded with the JSP-modified electrode.

Figure 5. Relationships between the absolute values of the bioelectrocatalytic current density and the background current density for (A) Vulcan, (B) JSP, (C) 1J9V, (D) 5J5V, and (E) 91V. Error bars indicate 90% confidence intervals for \(j_{\text{lim}}\) and \(j_b\) for each electrode.

Figure 6. Relationships between the absolute values of the bioelectrocatalytic current density and the background current density for (A) different amounts of Vulcan overlaid on the JSP layer (80 µg) and (B) various amounts of JSP overlaid on the Vulcan layer (80 µg).

Therefore, the accumulated JSP layer not only induces resistance to substrate transfer to the Vulcan layer but also to the electrochemically inactive insulation layer. The insufficient electrical contacts of the upper part of the JSP layer indicate that there is hardly any electrical connection between the JSP particles.

5. Conclusions

A theoretical model was constructed for analyzing the porous electrode surface as the scaffold of the DET-type bioelectrocatalysis. The porous structure of the electrode affected the substrate transfer and limited the mass-transfer-controlled current. Based on the model, the surface properties of the carbon composites constructed with JSP and Vulcan were compared for the scaffold of the DET-
type bioelectrocatalysis of BOD. The analysis suggests that the interface between the JSP and Vulcan particles is a more effective scaffold than the interface between the individual JSP particles or Vulcan particles. According to the orientational analysis of the adsorbed enzyme, there are many contact points between BOD and the electrode at the JSP/Vulcan interface than at the other interfaces (JSP/JSP or Vulcan/Vulcan).

Supporting Information

The Supporting Information is available on the website at DOI: https://doi.org/10.5796/electrochemistry.20-00128.

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