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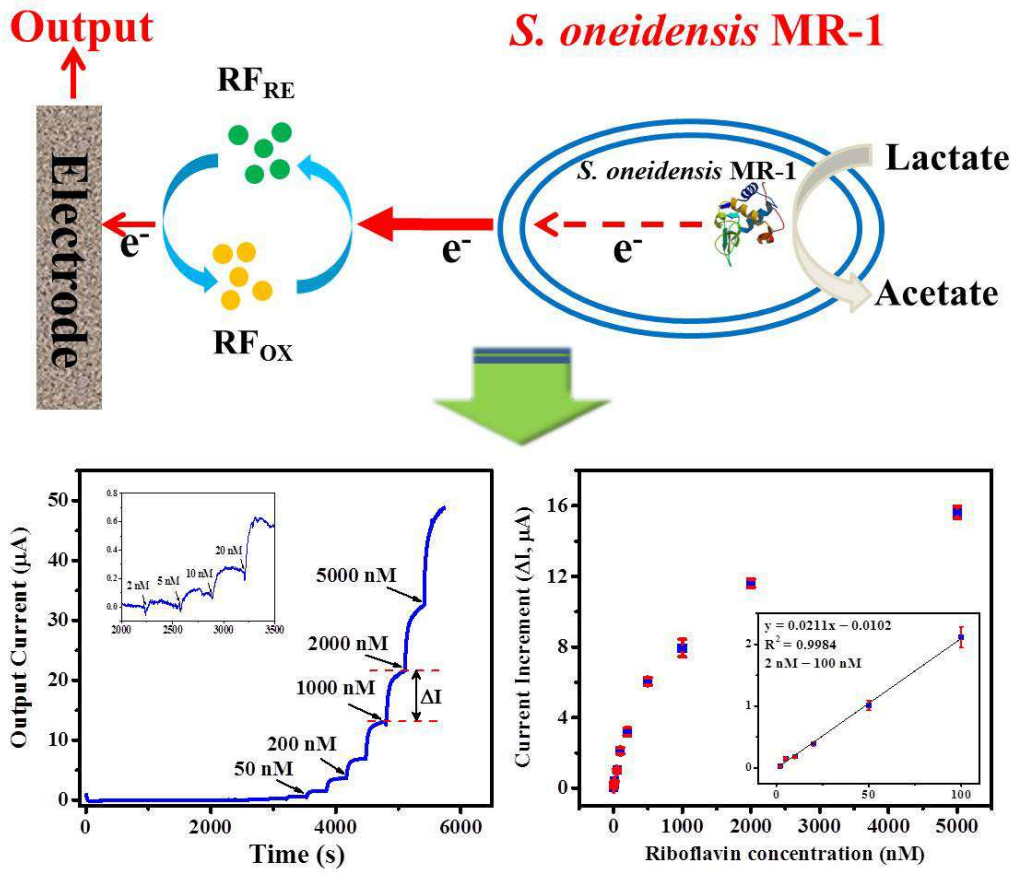

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Sensitive Amperometric Detection of Riboflavin with a Whole-Cell Electrochemical Sensor

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Abstract

A novel whole-cell electrochemical sensor was developed and applied for sensitive amperometric detection of riboflavin. In this work, a whole-cell based riboflavin redox cycling system was characterized, in which electroactive bacteria *Shewanella oneidensis* MR-1 was employed as the biocatalyst to regenerate the reduced riboflavin after the electrode oxidation. This redox cycling system efficiently enhanced the electrochemical response of riboflavin and enabled a stable current output at poised electrode potential. Thus, a sensitive amperometric biosensing system for riboflavin detection was developed by integrating this whole-cell redox cycling system with the conventional riboflavin electrochemical sensor. Remarkably, this riboflavin biosensor exhibited high sensitivity (LOD= 0.85 ± 0.09 nM, *S/N*= 3), excellent selectivity and stability. Additionally, reliable analysis of real samples (food and pharmaceutical samples) by this biosensor was achieved. This work provided sensitive and practical tool for riboflavin detection, and demonstrated that the integration of electroactive bacteria and using its outwards electron transfer for redox cycling would be a powerful and promising strategy to improve the performance of electrochemical sensing system.

Keywords: riboflavin; whole-cell; extracellular electron transfer; *Shewanella*; biosensor

1. Introduction

Riboflavin (also known as Vitamin b_2) is a well-known water soluble vitamin and is essential to the human health. It is the central module for coenzymes as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which play vital roles in series of carbohydrates, fats and protein metabolism [1]. Deficiency of riboflavin would result in various metabolic disorder and diseases like stomatitis and skin rashes [2]. Meanwhile, the excess riboflavin is also threatening to human health since it is an efficient photosensitizer and would induce oxidative damage to tissues and DNA when exposed to UV [3, 4]. More importantly, riboflavin cannot be produced in the human body, and has to be taken in from dietary source, supplements or medicines [2]. Thus, developing new strategies and techniques for efficient determination of riboflavin concentration is significative in forages, food, supplements quality control and clinical diagnoses.

Although most riboflavin detection techniques are depended on its distinctive fluorescence characteristic [5-8], the excellent redox property of riboflavin makes it applicable for electrochemical detection [9]. As a result, various electrochemical techniques have been developed for riboflavin determination [10-16]. Most of these riboflavin electrochemical sensors were based on the conventional voltammetry analyses including differential pulse voltammetry (DPV)[10, 11], cyclic voltammetry (CV)[12, 16] and square wave voltammetry (SWV)[17, 18], in which the response current variation upon the electrode potential change is recorded. Despite the subtle differences on the electrode potential control mode, these voltammetry sensors are

principally based on direct riboflavin transformation at electrode surface upon consecutive electrode potential change. As a result, most of these reported attempts were focused on electrode modification [11, 14, 16, 19, 20].

However, further improvement of the riboflavin detection performance through electrode modification is difficult. Firstly, the margin effect of electrode modification gradually becomes weak since riboflavin originally has excellent electrochemical activity and fast electron transfer rate [21, 22]. Secondly, the output signal in conventional riboflavin electrochemical sensor is limited by the local riboflavin concentration as it is irreversibly consumed during electrochemical measurement. As a result, new strategies should be developed and integrated into conventional riboflavin electrochemical sensor in order to improve its analytical performance [10, 13, 23].

On the other hand, coupling proper reactions to regenerate riboflavin after it is registered at electrode (via oxidation/reduction reaction) may lead to repeated response of single riboflavin molecule and enhance current output of respective riboflavin electrochemical sensor. The idea of developing such redox cycling system for signal amplification attracts vast research interests and accordingly a wide range of ultra-sensitive detections of diagnostic biomarkers, pathogens, toxic heavy metal ions and pesticides have been demonstrated [24-28]. Meanwhile, most of these redox cycling systems consisted of multiple enzymes and nanomaterials, coupled with series enzymatic, electrochemical and chemical reactions. Compared with enzymatic biosensors, whole-cell biosensors exhibit distinctive advantages as high stability, simple preparation procedures, and also reduced cost [29]. Thus, design and

construction of whole-cell based redox cycling system is valuable for high sensitive riboflavin detection.

Theoretically, the whole-cell based redox cycling system can be realized in two manners based on whether the redox species are recycled via cell oxidation or reduction. Dissimilatory metal-reducing bacteria *Shewanella oneidensis* is the promising candidate for such redox cycling as it is capable of bidirectional extracellular electron transfer [30, 31]. *Shewanella* cells could transfer the endogenous electron to the electrode via sequenced cytochromes including CymA, MtrA, MtrB and MtrC/OmcA and flavins when the positive electrode potential is poised (more positive than -0.4 V *vs.* SCE), known as outwards EET [32, 33]. Meanwhile, this process could also be reversed and finally reduce the fumarate at cell periplasm with cathodic electron when negative electrode potential as -0.6 V (*vs.* SCE) was poised, which is known as inwards EET [30]. Remarkably, the bidirectional electron transfer between *Shewanella* cells and electrode is controlled by the redox potential shift of flavin when it interacts with outer-membrane cytochromes (MtrC/OmcA)[34]. Thus it is feasible to design *Shewanella* based riboflavin redox cycling system. In our previous work, we developed a riboflavin cycling system based on *Shewanella* inwards electron transfer, in which negative potential (-0.6 V *vs.* SCE) was selected to allow riboflavin to be reduced by the electrode. The reduced riboflavin then transferred the electron to *Shewanella* cells via its inwards electron transfer and finally reduced fumarate to succinate with the aid of fumarate reductase at *Shewanella* periplasm. This electrode reduction and cell oxidation process enabled riboflavin cycling, which increased the

response current and allowed the development of a riboflavin amperometric sensor [23]. Here, we postulate that the *Shewanella* outwards electron transfer capability also can be adopted for riboflavin cycling system (by poising the electrode at positive electrode potential), in which the endogenous electron from *Shewanella* metabolism could be used to continuously reduce the electrode-oxidized riboflavin.

In this work, electroactive bacteria *S. oneidensis* MR-1 (served as the biocatalyst) and its outwards electron transfer capability was adopted to construct a whole-cell based riboflavin redox cycling system. CV analyses were conducted to study the effect of *Shewanella* cells and microbial endogenous electron on the electrochemical behavior of riboflavin. Meanwhile, the feasibility of using this redox cycling system integrated electrochemical cell for the stable amperometric discharge was investigated. Riboflavin amperometric sensor based on *Shewanella* outwards electron transfer was then developed. The detection range, sensitivity, selectivity and stability of this riboflavin amperometric sensor were studied. Furthermore, riboflavin in spiked authentic samples, pharmaceutical and food samples were tested using the developed sensing system to evaluate its potential application.

2. Materials and Methods

2.1. Bacteria strains and cultivation conditions

Shewanella oneidensis MR-1 (ATCC 700550) was cultivated in LB broth (10 g/L tryptone, 5 g/L yeast extract (Oxoid, UK), 5 g/L NaCl, pH=7.2) at 30 ^{o}C in a shaking incubator (ZWY-100H, Shanghai Zhicheng Analytical Instrument Manufacturing Co.,

Ltd., China) with 150 rpm [35, 36]. The bacteria were harvested by centrifugation with 5000 rpm for 10 minutes (Avanti J-E, Beckman Coulter, USA) and washed with M9 salt medium (1 g/L NH_4Cl, 0.5 g/L NaCl, 17.8 g/L $Na_2HPO_4 \cdot 12H_2O$, 3 g/L KH_2PO_4, 0.0111 g/L $CaCl_2$, 0.12 g/L $MgSO_4$, pH=7.0 [37]) for three times to remove bacterial metabolites and re-suspended in the fresh electrolyte (M9 salt medium with 10 mM sodium lactate as carbon source, bubbling nitrogen for 15 minutes before use) to desired density [38, 39]. All chemicals were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (China) unless otherwise indicated.

2.2. Electrochemical setup

All the electrochemical analyses were conducted with CHI660E electrochemical workstation (Chenhua Instruments Co., Ltd., Shanghai, China). Three-electrode electrochemical system assembled in a 15 mL cylindrical borosilicate glass bottle was adopted for electrochemical analyses and electrochemical biosensor measurement. Carbon cloth with geometry size of 1 cm × 2 cm was used as working electrode, a platinum wire electrode and saturated calomel electrode (SCE, +0.243 V *vs.* SHE)(Chenhua Instruments Co., Ltd., Shanghai, China) were used as counter and reference electrode, respectively. Bacteria suspended in fresh electrolyte (12 mL) were inoculated into the electrochemical cell for electrochemical characterization or biosensing. Cyclic voltammetry (CV) analyses were conducted from -0.8 V to 0.1 V with scanning rate of 5 mV/s. The amperometric analyses were conducted at the constant potential of 0 V with sample interval of 1 second. Constant slow rate magnetic stirring (200 rpm) was adopted during the electrochemical analyses. All the

potential described in this work is versus SCE unless otherwise indicated. To maintain the sterile condition during the analyses, borosilicate bottle, carbon cloth electrode, platinum electrode, stirrer, mediums and all the other glassware were autoclaved at 121 ^{o}C for 30 minutes. The SCE was sterilized by wiping with 70% ethanol and air dried in the biosafety cabinet before use. Then, the electrochemical cell was assembled in the biosafety cabinet and operated at tightly sealed condition to avoid contamination.

2.3. Riboflavin sensor construction and sample analyses

After bubbled the cell suspension with nitrogen for 15 minutes, the amperometric analytical system was set-up using the above three-electrode system with a poised potential (0 V). The system was first discharged without riboflavin addition to enable baseline current response. The riboflavin (Sigma-Aldrich, USA) contained water samples or real samples (60 μL) were then spiked into the system and the corresponding current increments were monitored. The riboflavin concentration depended current response was studied by consecutively adding riboflavin contained water samples from 2 nM to 5 μM with an interval of 300 seconds. Linear fitting of the current increment with corresponding riboflavin concentration was then conducted to calculate the detection sensitivity of the riboflavin electrochemical sensor. The biosensor selectivity was tested by adding a group of vitamins (vitamin b_1, b_3, b_6, b_9 and b_{12} (Biosharp, China), 100 μM for each vitamin sample) and mineral salts ($FeCl_3$, $CaCl_2$ and $MgCl_2$, 1 mM for each mineral salt) mixture after riboflavin (100 and 500 nM) addition in consecutive amperometric analyses. The biosensor storage stability

was investigated by comparing the performance of biosensors which were preserved in the 4 ^{o}C refrigerator for different time period (10 days in total). Vitamin tablet, B complex tablet (By-Health Co., Ltd., China) and milk powder (Beingmate Co., Ltd., Hangzhou, China) were adopted as the typical standard sample for real sample measurement. To minimize the influence of temperature fluctuation, all the electrochemical analyses were conducted in a temperature-controlled room (30 ± 2 ^{o}C).

2.4. Riboflavin quantification with HPLC

Riboflavin quantification with HPLC was performed as described elsewhere [40] using an Agilent HPLC system (Agilent 1290, Agilent, USA) equipped with Agilent C18 analytical column (4.6 mm × 150 mm, 5 μm particle size) and a fluorescence detector. Samples were filtrated through a 0.22 μm filter before injection.

3. Results and Discussion

3.1. Riboflavin cycling with endogenous electron of *Shewanella*

In most conventional riboflavin electrochemical sensors, the riboflavin is irreversibly oxidized at electrode surface and one-off output signal is thus generated (Fig. 1a). Since riboflavin is the essential electron shuttle of *Shewanella* EET [41-43], it would be theoretically applicable to utilize *Shewanella* cells to charge the electrode-oxidized riboflavin with the endogenous electron released from lactate metabolism. This charging process might enable riboflavin repeated registration and amplify the electrochemical response (Fig. 1b). Accordingly, a novel whole-cell based

193 riboflavin redox cycling system was developed by dispersing *Shewanella* cells in the
194 electrolyte for three-electrode system (Fig. S1).

195 To verify the feasibility of applying *Shewanella* cells and its outwards electron
196 transfer for riboflavin redox cycling, CV analyses were conducted in this *Shewanella*
197 integrated three-electrode system, together with bare electrode (only riboflavin and
198 electrode) and riboflavin free (only *Shewanella* and electrode) control (Fig. 2a).
199 Magnetic stirring (200 rpm) was applied to improve riboflavin diffusion. When only
200 riboflavin was added in the system, the corresponding CV curve (red curve) showed a
201 pair of reversible peaks centered at around -0.46 V (Fig. 2a), which is the typical
202 electrochemical characteristic of riboflavin at neutral conditions [10, 41, 44]. The
203 *Shewanella* cells alone did not show obvious CV peak, indicating the residual
204 riboflavin concentration in the *Shewanella* suspension was low (Fig. 2a). Impressively,
205 the CV curve of riboflavin with *Shewanella* cells and lactate transformed to the typical
206 sigmoid anodic curve with midpoint potential of -0.46 V (consistent with the CV
207 response of riboflavin) and was fitted with Monod kinetics (blue curve, Fig. 2a)[45].
208 The evolution of riboflavin CV curve from symmetrical peak to sigmoid shape
209 indicated that stable anodic discharge via riboflavin was established, which was the
210 evidence of the continuous microbial recharging of riboflavin after its electrode
211 oxidation (Fig. 1b) [46]. Meanwhile, the height of peak current (i_P) with *Shewanella*
212 cells was higher than that only with riboflavin (7.1 $\pm$ 0.4 *vs.* 2.0 $\pm$ 0.1 μA).

213 Furthermore, the sigmoid shape of the CV curve implies a stable anodic
214 amperometric response to riboflavin might be obtained at positive electrode potential

(more positive than -0.4 V)[47]. Compared with voltammetry analyses, amperometric analysis showed tremendous advantages for biosensor design [48]. Thus, the amperometric response of riboflavin under different conditions (with or without *Shewanella* cells) was compared (Fig. 2b). Based on the CV analyses (Fig. 2a), an electrode potential of 0 V was selected to guarantee fully riboflavin oxidation and retain the small background current of direct electron transfer via *Shewanella's* OM cytochromes [49]. According to the i-t curve monitored, strong current increment (~1.3 μA, 100 nM riboflavin) upon riboflavin addition was obtained in the electrochemical cell with *Shewanella* cells (blue curve), while only weak response (~2 nA, 100 nM riboflavin) with large fluctuation and fast decay to riboflavin was observed in that without *Shewanella* cells (red curve). Furthermore, the response current soon became stable, indicating riboflavin can quickly reach redox equilibrium during amperometric discharge and microbial recharging. It is worth to note that *S. oneidensis* MR-1 is also able to produce low level of riboflavin. However, no significant riboflavin production could be detected during the amperometric detection process by HPLC analysis, which was due to the poor nutrient of electrolyte (M9 mineral salt medium supplemented with sodium lactate) and the short test time (around 10 minutes per sample). Moreover, the current increment (ΔI) to exogenously added riboflavin sample was calculated based on baseline extraction, which also could exclude possible interference of accumulated microbial produced riboflavin. Therefore, the influence of the microbial riboflavin production could be eliminated. These results implied that sensitive amperometric detection of riboflavin is applicable by applying *Shewanella*

cells and its endogenous electron for riboflavin redox cycling.

3.2. Biosensing system development and optimization

Next, a whole-cell electrochemical biosensing system for amperometric detection of riboflavin was developed.

Since *Shewanella* cell was adopted as the biocatalyst, the operation condition, cell density and physiological status would influence the *Shewanella* electrochemical activity and thus biosensor performance. According to previous work, *Shewanella oneidensis* MR-1 is a kind of mesophilic bacteria with optimal growth temperature around 30 ^{o}C [50] and optimal pH of about 7.0 [51], the electrochemical sensor was thus operated in the temperature-controlled room (30 $\pm$ 2 ^{o}C) with phosphate buffer contained electrolyte (pH= 7.0) to maintain the neutral pH during analyses. The cell density optimization was conducted by comparing the amperometric response (1 μM riboflavin) of the biosensor with cell density ranging from OD_{600}= 0.05 to 2. The biosensor with cell density of OD_{600}=1.5 (~1.5 ± 0.1×10^{8} CFU/mL) performed superior than other cell density (Fig. S2a). To study the influence of cell physiology, *Shewanella* cells with different cultivation time (5 hours to 20 hours) were harvested and re-dispersed to OD_{600}=1.5. The biosensor performance with bacteria from different growth stage was then compared. It was found that biosensor with cells cultivation time of 16 hours showed highest response (Fig. S2b). Thus, temperature of 30 ^{o}C, pH of 7.0, OD_{600}= 1.5 and cultivation time of 16 hours were fixed for the later experiments.

The riboflavin concentration depended response was determined by consecutively adding riboflavin (2 nM ~ 5000 nM) to biosensor system with an interval of 300 seconds. As shown in Fig. 3a, a step-wise current increment with noteworthy concentration dependence was observed under successive injection of riboflavin. A linear dependence with a high sensitivity of 0.0211 ± 0.0023 μA/nM at low concentration range (2 nM ~ 100 nM) was determined (y= 0.0211x – 0.0102, R^2= 0.9984). When the added riboflavin concentration was over 100 nM, there was a decrease in the current increment to riboflavin concentration. The limit of detection (LOD) was estimated to be 0.85 ± 0.09 nM by equation: $C_L=3\delta/S$ (at a signal-to-noise ratio of 3, S/N= 3), where δ is the standard deviation of blank samples, S is detection sensitivity (slope of calibration curve) [52, 53]. The detection sensitivity (21.1 nA/nM) and LOD (0.85 nM) are superior to most of the recently reported riboflavin electrochemical sensors (Table S1). Meanwhile, most of these previous riboflavin sensors relied on sophisticated electrode modification (like nanoparticles/nanotubes, graphene, ionic liquid and their composite) to improve the electrochemical response of riboflavin [12, 14, 19, 20, 53, 54]. These designs suffer from the high cost (for example, noble metal catalyst like gold nanoparticle may be used) and tedious preparations (multiple procedures required to modify the electrode with composite material). More importantly, riboflavin was irreversibly registered in these modified electrode and the thus a relative low sensitivity were reached. In contrast, adoption the biocatalyst of *Shewanella* cells reduces the cost and helps to establish a riboflavin redox cycling system, which enables the repeated registration of riboflavin and

improved the sensitivity. Furthermore, It is also worth to note that the analytical performance obtained here just based on conventional electrode (bare carbon cloth), which might be further improved with sophisticated electrode modification. These results proved that integrating *Shewanella* cells and its outwards electron transfer to conventional riboflavin electrochemical sensor enables amperometric detection of riboflavin, and significantly improves the riboflavin biosensing performance.

3.3. Analytical performance and Application in real samples

The selectivity, stability, accuracy and reproducibility of the developed biosensor were investigated before it was applied for real sample measurement.

Riboflavin analogues (vitamin b_1, b_3, b_6, b_9 and b_{12}, with 100 μM for each sample) and common mineral salts ($FeCl_3$, $CaCl_2$ and $MgCl_2$, 1 mM for each sample) existed in food samples were chosen as possible interferences to evaluate the selectivity/interference resistance of this biosensor. It was observed that interferences addition did not result in significant current change compared with the control (baseline current) (Fig. S3), which indicated that this biosensor showed good selectivity to riboflavin. Meanwhile, the amperometric current response (current increment from baseline) to riboflavin (1 μM) with mixed interferences (8.6 μA) showed no significant difference with that of riboflavin alone (8.9 μA), which substantiated that this biosensor had excellent interference resistance.

The stability is an important consideration for potential application of biosensor. The stability of this developed biosensor was evaluated by comparing the individual

303 current output of parallel prepared biosensors with different storage duration (at 4 ^{o}C
304 refrigerator). It can be found that low temperature storage did not significantly reduce
305 the biosensor activity (Fig. 4). Biosensor output signal slowly decayed during the
306 continuous storage and around 85% output was retained after storing at 4 ^{o}C for 10
307 days. The accuracy and reproducibility were investigated by analyzing the water
308 samples spiked with authentic riboflavin (Table 1). It could be found that the biosensor
309 quantification results were consistent with spiked concentration. For water sample
310 with riboflavin concentration from 10~120 nM, the recovery obtained by this
311 biosensor assay was between 90.0% and 97.5%, implying the biosensor has good
312 accuracy. Meanwhile, the corresponding coefficient of variation was between 1.9%
313 and 6.2%, indicating the good reproducibility of the biosensor. These results indicated
314 that as developed biosensor is highly reliable for the determination of riboflavin in the
315 aqueous samples.

316 Since the riboflavin analysis is of great significance both in food industry and
317 medical application, vitamin tablet, B complex tablet and milk powder were selected
318 as the representative real samples for biosensor application. The riboflavin
319 concentration determined by the developed biosensor was compared with HPLC
320 results and manufacture's declaration (Table 2). It was found that the quantification
321 results determined by the developed biosensor were consistent with that obtained by
322 HPLC analysis and manufactures' declaration. Moreover, possible interferences in
323 these real samples did not significantly affect the detection accuracy, although the
324 ingredients of these samples are quite complicated. These results suggested that the

biosensor developed here was robust enough for real applications.

Conclusion

In this work, *S. oneidensis* MR-1 cell was integrated into riboflavin electrochemical system and its outwards electron transfer capability was adopted for riboflavin redox recycling, in which endogenous electron generated from *Shewanella* lactate metabolism was applied for the regeneration of reduced riboflavin. Based on this integrated system, a sensitive amperometric biosensor for riboflavin detection was further developed. The developed biosensor showed low LOD, high sensitivity, excellent stability and was successfully applied for real sample measurement. This work demonstrated the promise for utilizing its outwards electron transfer for redox cycling and signal amplification, which might add new concept for biosensor design.

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489
490

Table 1. Analysis of water samples spiked with authentic riboflavin.

Riboflavin spiked (nM)	Determined by biosensor (nM)	COV (%)*	Recovery (%)
10	9.71	3.9	97.1
50	46.9	5.4	93.8
80	7	5.6	96.2
100	97.5	6.2	97.5
120	107.9	1.9	90.0

* COV: coefficient of variation (n= 3)

503 **Table 2.** Analysis of riboflavin in commercial pharmaceutical and food samples.

Sample	Claimed by manufacture (mg)	Determined by HPLC (mg)	Determined by biosensor (mg)	COV* （%）	Recovery （%）
Vitamin tablet	1.4	1.3	1.3	11.4	92.9
VB complex tablet	4.2	4.2	4.3	5.9	102
Milk powder	0.63	0.60	0.57	12.8	90.4

504 * COV: coefficient of variation (n= 3)

505

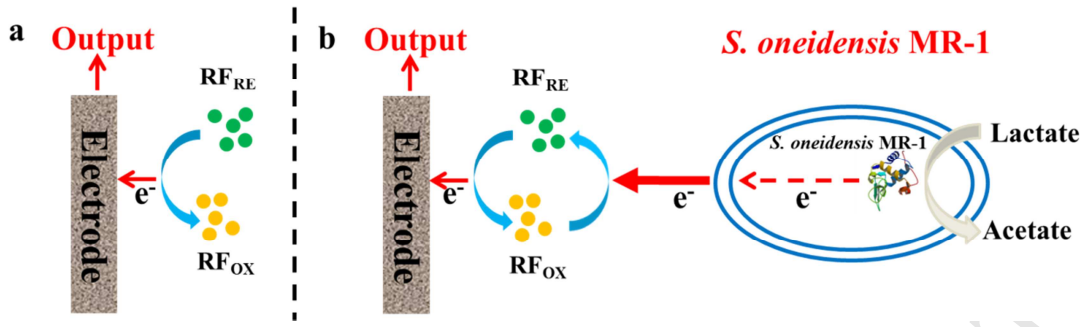
Figure Legends

Figure 1. Schematic of (a) conventional electrochemical system for riboflavin detection; (b) *Shewanella* cells (biocatalyst) integrated riboflavin electrochemical sensing system developed in this study. RF_{RE} indicates reductive riboflavin, RF_{ox} indicates oxidative riboflavin.

Figure 2. (a) Cyclic voltammetry curves of riboflavin, *Shewanella* cells, and *Shewanella* cells with riboflavin (riboflavin addition is 500 nM, scanning rate is 10 mV/s); (b) Amperometric response of riboflavin with or without *Shewanella* cells (working electrode was poised at 0 V). Arrows indicates riboflavin injection. Inset is the enlarged view of the selected area.

Figure 3. (a) Biosensor current output with consecutive addition of riboflavin (baseline was subtracted); arrows indicates riboflavin injection. (b) Current increment with increased riboflavin concentration and corresponding calibration curve. Inset is the enlarged view of low concentration range. The error bars were calculated from three individual experiments.

Figure 4. The storage stability of the developed biosensing system. The error bars were calculated from three individual experiments.



526

527 **Figure 1.**

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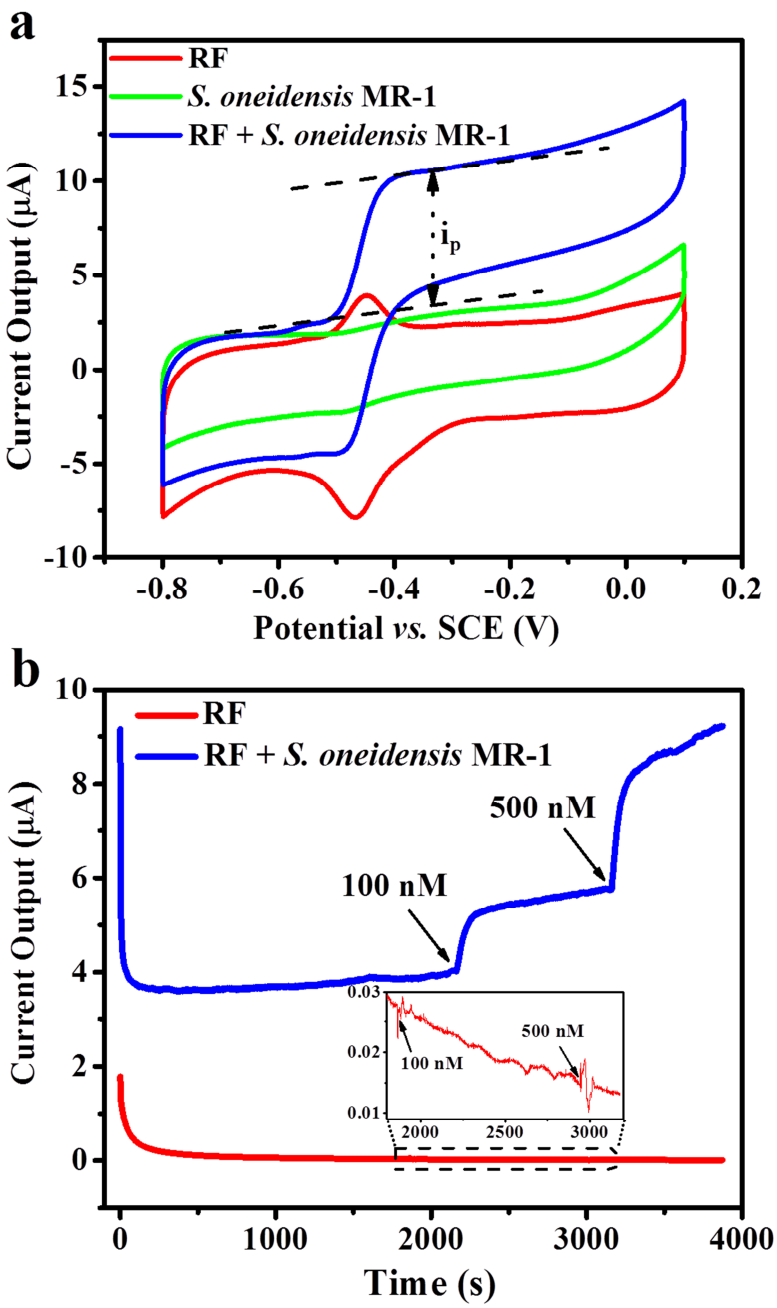


529

530 **Figure 2.**

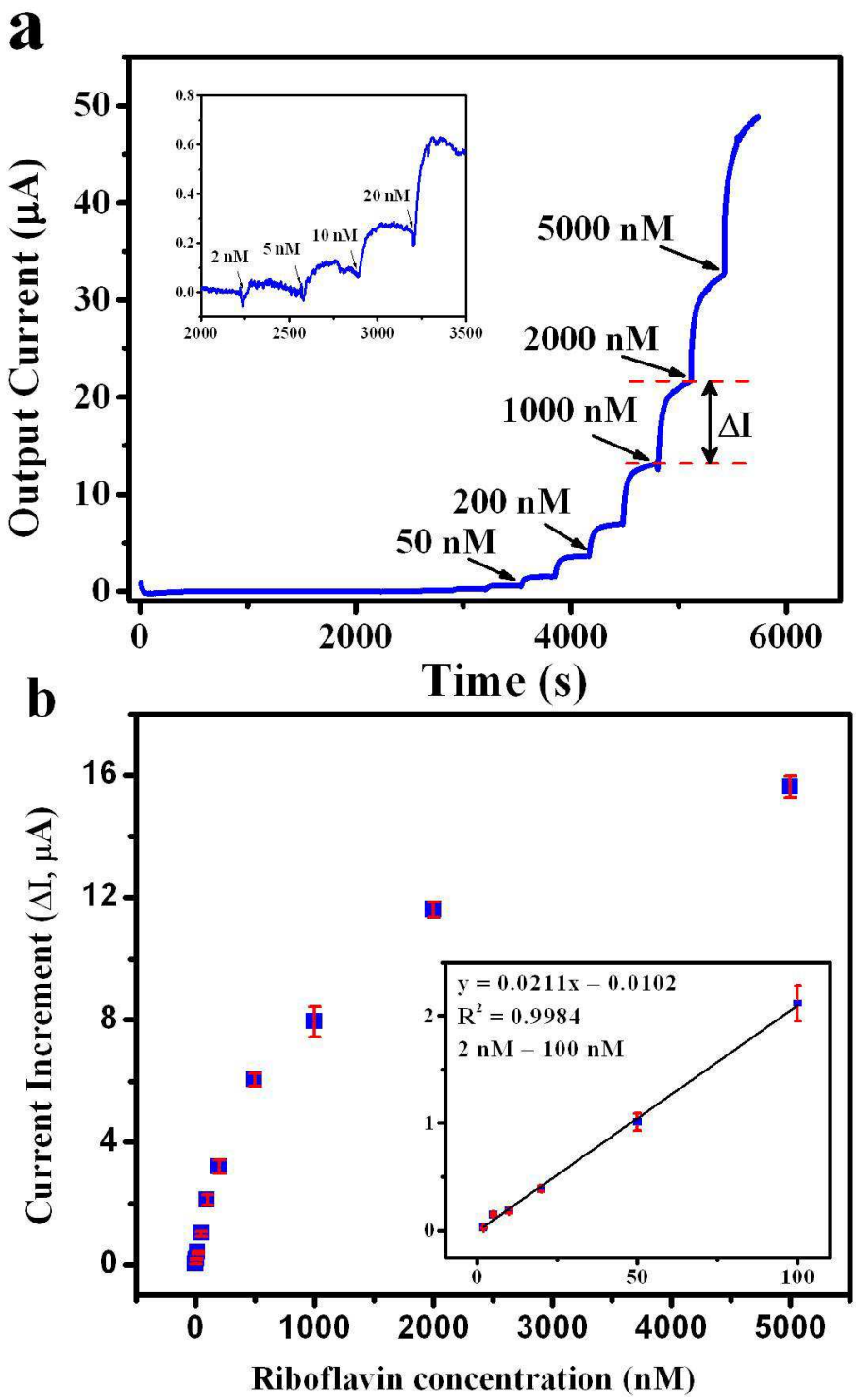
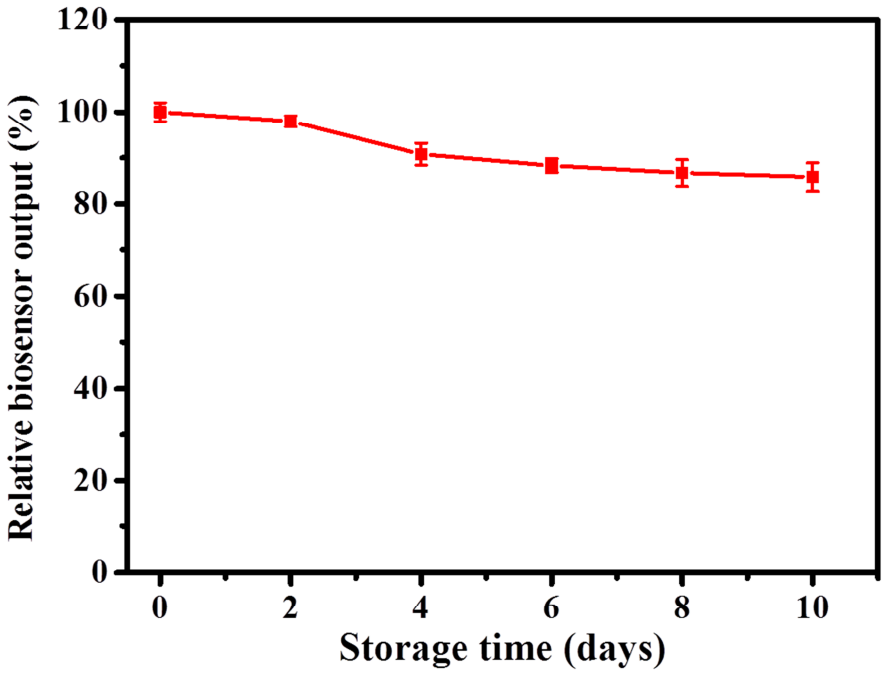

a
Output Current (μA)
Time (s)
2 nM
5 nM
10 nM
20 nM
50 nM
200 nM
1000 nM
2000 nM
5000 nM
ΔI
b
Current Increment (ΔI, μA)
Riboflavin concentration (nM)
y = 0.0211x – 0.0102
R² = 0.9984
2 nM – 100 nM

Figure 3.



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535 **Figure 4.**

Highlights

- Riboflavin cycling system based on *Shewanella oneidensis* and its outwards electron transfer was investigated.
- An amperometric biosensor with *Shewanella* endogenous electron was developed for riboflavin detection.
- A low LOD (0.85±0.09 nM) and high sensitivity (0.0211±0.0023 μA/nM) was achieved.

Supporting information

Sensitive Amperometric Detection of Riboflavin with a Whole-Cell Electrochemical Sensor

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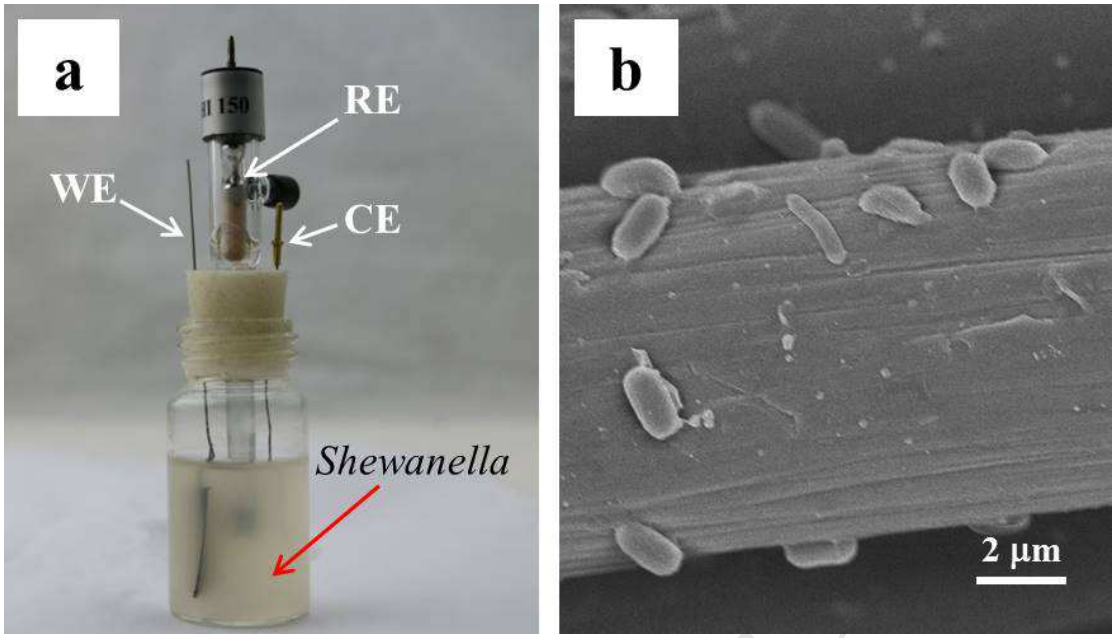

Figure S1. (a) Photograph of *Shewanella* cells integrated riboflavin amperometric sensing system. The red arrow indicates the turbidity caused by planktonic *Shewanella* cells which acted as biocatalyst for riboflavin recharging; WE: working electrode (carbon cloth electrode); RE: reference electrode (saturated calomel electrode, +0.243 V *vs.* SHE); CE: counter electrode (platinum wire electrode). (b) SEM images of the carbon cloth (WE) after biosensor disassembly, indicating certain amount of *Shewanella* cells attached to the electrode surface during amperometric analysis.

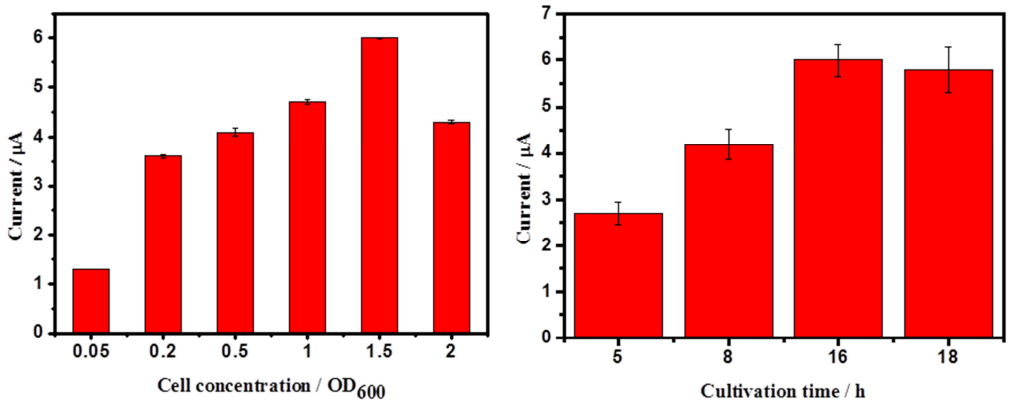

Figure S2. Influence of (left) bacteria cell concentration and (right) bacteria cultivation time on biosensor current output (baseline current was subtracted). 1 μM riboflavin was added.

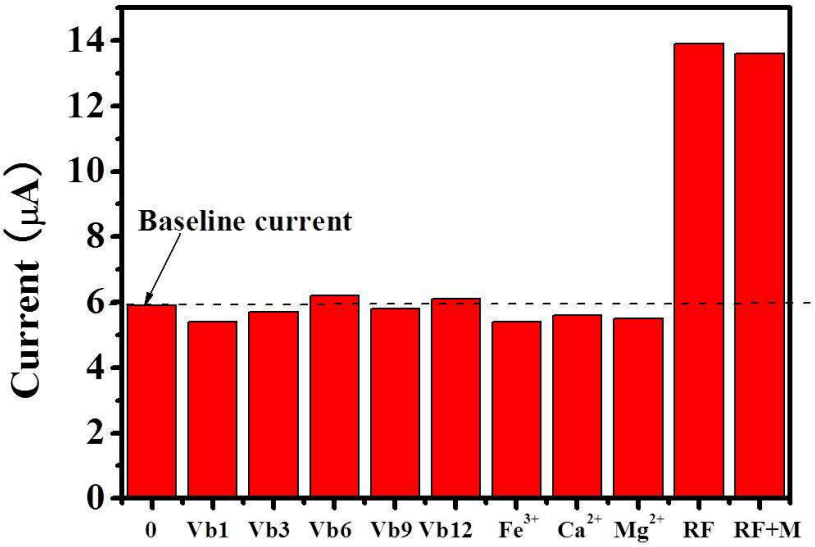

Figure S3. Interference influence on the biosensor current output. '0' in X-axis means no addition, and the current was considered as the baseline. 'RF+M' means riboflavin (1 μM) with mixed interferences (contains all vitamins and mineral salts listed below). Vitamin b_1, b_3, b_6, b_9 or b_{12} added was 100 μM, mineral salt ($FeCl_3$, $CaCl_2$ or $MgCl_2$) added was 1 mM, riboflavin added was 1 μM.

Table S1. Comparison of recently reported riboflavin electrochemical sensors.

Electrode[#]	Modification	EC method*	LOD	Linear range	Sensitivity $nA \cdot nM^{-1}$	Ref.
PGE	dsDNA	DPV	0.9 mM	1.3 mM – 0.19 mM	**3.08×10^{-3}**	[1]
GCE	p-AMTa film	DPV	45.4 nM	10 μM – 90 μM	**0.11**	[2]
GCE	Cr doped SnO_2 NP	DPV	0.11 mM	0.2 mM - 0.1 mM	**0.047**	[3]
GCE	OLA-NiO-MWCNT	DPV	1 nM	9 nM - 55.9 mM	**0.489**	[4]
GCE	PT nanotube	DPV	3 nM	10 nM – 65 μM	**1.03**	[5]
Gold	ssDNA-MoS_2-Graphene	DPV	20 nM	25 nM – 2.25 mM	**0.83**	[6]
GCE	dsDNA-AuGNP-MWCNT-αFe_2O_3	LSV, SWV	6 nM	6 nM – 0.3 mM	**4.0**	[7]
CP	MnO_2	DPV	15 nM	20 nM – 9 μM**	**N/A**	[8]
CC	None	AMP	2.2 nM	5 nM – 100 nM 100 nM – 10 μM	**85** **41**	[9]
GCE	MWCNT/[BMPi]PF_6	DPV	4.7 nM	26 nM – 1.3 μM	**8.27**	[10]
CC	None	AMP	0.85 nM	2 nM – 100 nM	**21.1**	This work

[#] PGE: pencil graphite electrode; GCE: glassy carbon electrode; CP: carbon paste; CC: carbon cloth

*DPV: differential pulse voltammetry; SWV: square wave voltammetry; LSV: Linear scanning voltammetry; AMP: amperometry

**linear with logarithm of concentration

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