Microbial community analysis during start-up of anaerobic co-digestion based on quinone profiles using supercritical fluid extraction

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Abstract. Quinone profile is well known as a useful tool for the analysis of microbial community dynamics in mixed cultures in terms of quantification, simplicity, and reproducibility. The application of quinone profile method in anaerobic digestion is to monitor and overcome instability during fermentation process. A lab-scale anaerobic digestion treating a mixture of milk cow manure (CM) and simulated food waste (FW) during start-up process at mesophilic conditions was used to monitor the change of microbial community dynamics and stability. Supercritical fluid extraction (SFE) experiments using CO_2 and ultra-high performance liquid chromatography (UPLC) was applied for extract and determination of ubiquinones (UQ) and menaquinones (MK) species. Quinone can be a helpful tool to make the link between microbial community and anaerobic digestion parameters in order to overcome digester instability during the start-up process.

Key words: Microbial community, anaerobic, super critical fluid extraction, quinone.

Introduction

Microbial community structure is one of the important factors controlling the pollutant-degrading capacity of ecosystems. The capacity of an ecosystem to degrade organic compounds and its response to the changes in environmental conditions depend not only on the total population of microorganisms present, but also on the microbial community structure of that system (Hu et al.., 2001). Conventional techniques that involve microbial enrichment and isolation are useful tools when studying specific culturable microorganisms. However, since most microorganisms in the environment are not culturable (Amann et al.., 1995), the cultivation-based methods may not be suitable for applications in evaluating the dynamic microbial community structure in environmental samples. Different advantages and limitations were found by using another methods. Molecular techniques using PCR based on rDNA such as denaturing gradient gel elctrophoresis (DGGE) and restriction fragment length polymorphism (RFLP) have not yield information on microbial biomass because the copy number of rDNA in each bacteria species is different (Farrelly et al.., 1995).

The fluorescent in situ hybridization (FISH) technique as a molecular analytical technique based on the enumeration of bacteria in various environments has been developed (Amann et al.., 1995). However, the FISH technique for analysis of a microbial community requires much time and a skilled operator to get reliable results. On the other hand, techniques of quantitative chemical analysis such as phospholipids fatty acid (PLFA)-profiling and microbial quinone-profiling have a high correlation with the biomass. The profile of PLFA does not represent individual taxonomic groups (Katayama and Fujie, 2000). Quinone profile is well known as a useful tool for the analysis of microbial community dynamics in mixed cultures in terms of quantification, simplicity, and reproducibility. Quinone profile method entails direct analysis of respiratory quinones in cell membranes to quantitatively reveal community profiles according to quinone molecular types (Yan et al.. 2002). It is superior to molecular technologies because it correlates quantitatively to the microbial biomass. It also gives more information on taxonomy compared PLFA method, because most of the microorganisms contain a major quinone species (Tang et al.. 2004).

However, until now, the application of quinone profile method in anaerobic digestion process has not been reported. In order to effectively control the anaerobic process, it is necessary to understand the microbial community structure and its change, especially its special role in decomposition of organic matters. Therefore, in this study, we aimed to

investigate the applicability of Quinone Profile as chemical biomarker in Methane Fermentation Process to control the microbial community dynamics and stability assessment of a laboratory scale anaerobic digester treating a mixture of milk cow manure and simulated food waste during the start-up process at mesophilic conditions.

Supercritical Fluid Extraction (SFE), using a green solvent: CO2 was performed to extract the bacterial quinone with a rapid and accurate ultra high performance liquid chromatography analysis (UPLC) for the identification and quantification of species.

Materials and Methods

Apparatus

A continuous, laboratory scale digester (EYELA JAR Fermenter MBF-800ME) was used for the anaerobic digestion. The volume was maintained at 6L with stirring rate during all the experiments was 150 rpm. The digester was inoculated with sludge from an industrial-scale anaerobic digestion plant treating cow manure. The temperature was maintained at mesophilic conditions 38°C (±1), and the initial organic loading rate was 19.2gVS/$l^{-1}d^{-1}$.

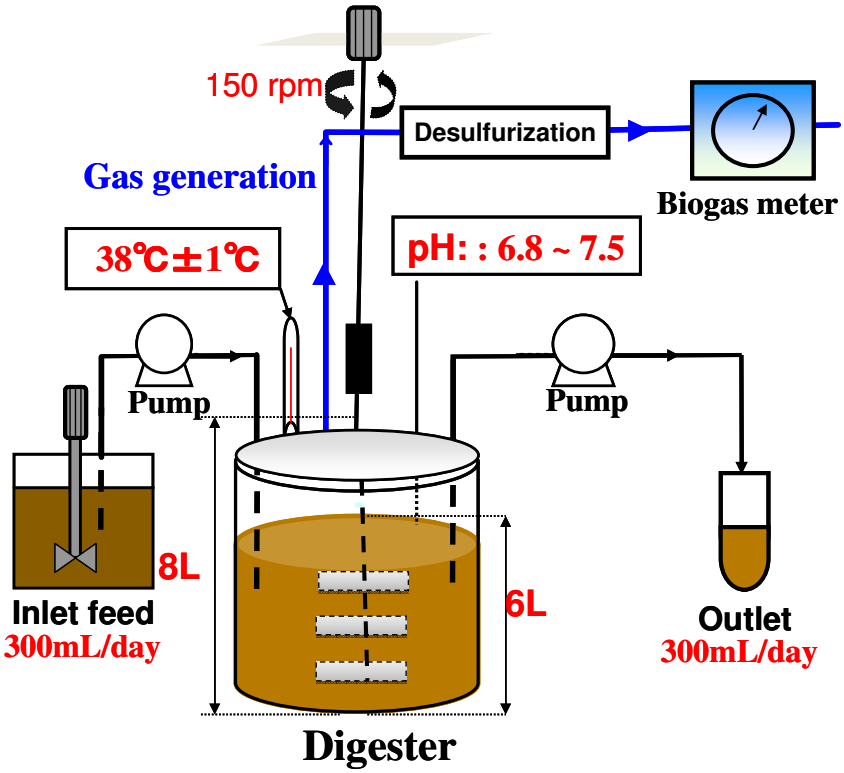

Figure 1. Scheme of the Digester

The substrate was fed everyday using a time-controlled pump with hydraulic retention time (HRT) of the digester was maintained to 20 days and biogas produced in the digester was measured daily. A detailed scheme of the digester is shown in Figure 1.

Feedstocks

Milk cow manure was obtained from Nakajima farm at Handa city in Aichi Prefecture. After collection straw was removed and the residue was stored at -20◦C. The composition of the simulated food waste is presented in Table 1. The simulated food waste was homogenized with a commercial blender and stored at -20°C.

Table 1. The Composition of Simulated Food Waste (percent in wet base)

Food	Composition	Ratio	Weight (g-wet)
	Apple	15	0.75
Fruit	Orange	10	0.50
	Cabbage	12	0.60
Vegetables	Potato	12	0.60
	Carrot	12	0.60
	Meat	7	0.35
Meat and Fish	Fish	7	0.35
	Rice	12.5	0.63
Staple food	Bread	12.5	0.63
Total		100	

The milk cow manure was diluted with pure water to obtain a solids level similar to that of unthickened milk cow manure. Both substrates were mixed at a preliminary determined ratio before feeding in the reactor as described in Table 2.

Table 2. Characteristics of Injection Sample Feed

MC FW (wet weight)	100:100	95: 05	90 : 10	80 : 20	70 : 30	50 : 50
Period (days	1 - 15	16 – 20	21 - 24	25 - 33	34 - 36	37 -63
Carbon conc. (g-C/L)			33			
HRT (days)			20			

Analytical techniques

Five consecutive interventions were implemented by incorporating step by step the proportion of simulated FW, from 5% to 50%, with organic loading rate of 19.2 $gVS/l^{-1}d^{-1}$ to 20.7 $gVS/l^{-1}d^{-1}$; in the digester in order to increase the biogas production and control the change in the microbial community using quinone profiles. Calcium bicarbonate was added occasionally to maintain the alkalinity level.

The pH was measured by a digital pH meter, the substrate and biomass concentrations were respectively determined in terms of the chemical oxygen demand (COD), total solids and volatile solids. Biogas production was measured everyday and composition was analyzed using a gas chromatograph (Shimadzu GC-8A).

All experiments samples were performed using a Supercritical Fluid Extraction (SFE) using CO_2 and methanol as modifier. Under the appropriate conditions, quinones were extracted and analyzed using an Ultra-high performance liquid chromatography (UPLC) equipped with column BEH C18, φ1.7μm, 2.1 x 150mm. A mixture of methanol and di-isopropyl ether (97:3, v/v) was used as the mobile phase at a flow rate of 0.5 ml/min.

Results and Discussion

Total Solids (TS) and Volatile Solids (VS)

The total solids (TS) concentration of the substrate influences the pH, temperature and effectiveness of the microorganisms in the decomposition process. This relationship also shows that a slight increase in the percentage of total solids with an increase in volume of biogas produced as shown in Figure 2. An increase of TS decreases amount of water, thus reducing the level of microbial activity represented by VS profile, which then affects the amount of biogás produced.

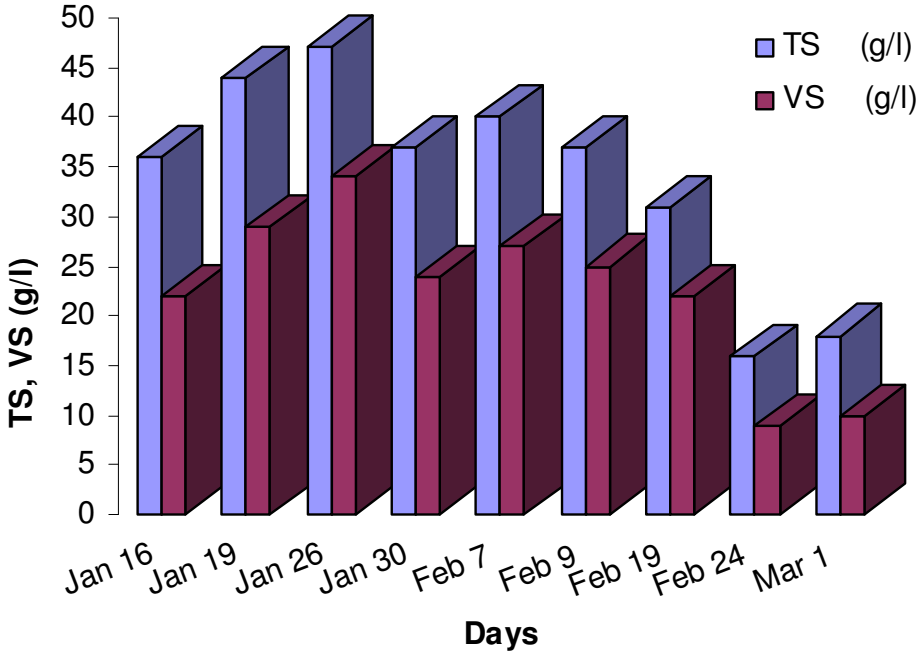

Figure 2. Total Solids (TS) and Volatile Solids (VS) Concentrations

Organic Acids

All methane gases produced in anaerobic digestion come from the volatile fatty acids. Acetic acid alone contributes to about 72% of the total production, propionic acid about 13% and other intermediate acids about 15% (McCarty, 1964). The fatty acids are formed as a result of breakdown of organic matter and played an important role in anaerobic digestion. In this experiment, acetic and propionic acids were the only major acids present in the reactors during all operating days. Other intermediate acids such as formic and butyric acids were not detected. Figure 3 shows only acetic acid is detected from day 23rd.

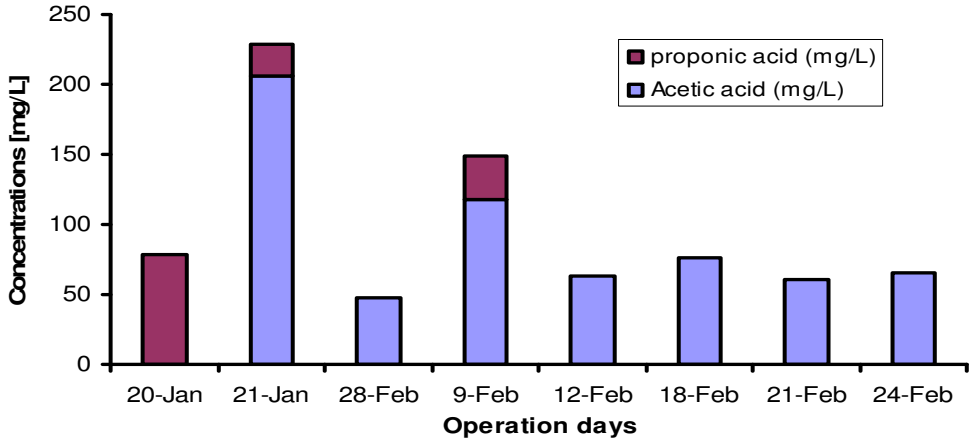

Figure 3. Organic acids Concentrations during digestión period

These acids act as the substrates for the bacteria that degrade them to methane. Thus the production of fatty acids is an important intermediate step in the overall anaerobic digestion process. A variation in the production of these fatty acids affects the methane formation and ultimately the degree of waste stabilization. The drop of acetic acids indicated their high utilization by methanogens. Irene S. et al.. reported that propionic acid has a stronger inhibitory effect on methanogenesis than acetic or butyric acid.

Biogas Production

Anaerobic co-digestion of cow manure (CM) and simulated food waste FW was carried out in three steps. Initially, the organic loading of the reactor was run with 100% CM and from Jan 20th, FW is added progressively from 5% to 50%, this is the transition phase. In the last phase, CM:FW ratio was maintained at 50:50 until the end of the experiment. These three phases are shown in Figure 4.

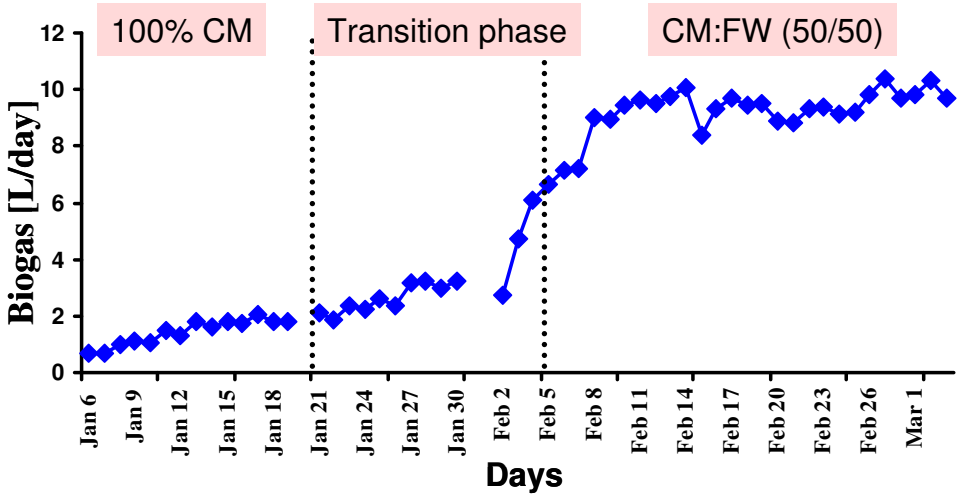

Figure 4. Biogas Production during digestión

At the first stage, the biogas yield was already around 2 l/d. The start-up period of adding FW was characterized by a parallel increase of the biogas production and volatile solids (VS) removal as the organic loading increased, showing a good acclimation of sludge to simulated FW during 15 days of the transition phase. The biogas production increased two times compared with the first stage. The maximum biogas production was observed at the last stage with 50% FW and 50% CM where the biogas yield reached more than 10 l/d. This biogas production was characteristic of the conversion of easily degradable substrates, mainly volatile fatty acids initially present in the manure.

The decrease in the biogas production and total VS removal confirmed that the maximum organic loading had been reached. The improvement in biogas yield seems to be related to the high biodegradability of FW added as co-substrate. The large biodegradability of this substrate was proven when digesting 50% of FW and resulted in the highest biogas yield. The biogas composition analyzed is presented with 64% of methane and 36% of Carbon dioxide.

Quinone Analysis

Microbial community structure during the start-up of anaerobic co-digestion of CM and FW were obtained by quinone profile analysis as shown in Figure 5. Quinone profiles changed quickly from 1.6 to 2.5 µmol/l as the FW is added step by step in the digester from 5% to 50%. The maximum amount of quinone (2.6µmol/L) was observed on February 8th with the ratio cow manure to food waste (50:50). However, the quinone amount started to decrease on day February 9th before reaching the stability from March 2nd.

The UPLC analysis detected and identified presence of eight types of quinones class, three ubiquinone species (UQ-8,-9,-10) and five menaquinones species MK-5,-6,-7,-8,-9. The mean value of UQ/MK ratio was around 0.05 (less than 1), therefore according to Hu et al., 2001, this value suggest that anaerobic bacteria were dominant with menaquinone as the major species and among them MK-7 still the dominant species, while for ubiquinone, UQ-9 was the dominant species However, in some analysis, ubiquinones were detected at a small quantity in some sample and sometimes some species were not detected like at January 14th, 23rd, 26th and February 18th. Also, for menaquinones species, MK-9(H4) was not detected on January 23rd. This can be explained by the partial degradation of the substrate by the microbial community as we used a continuous digestion.

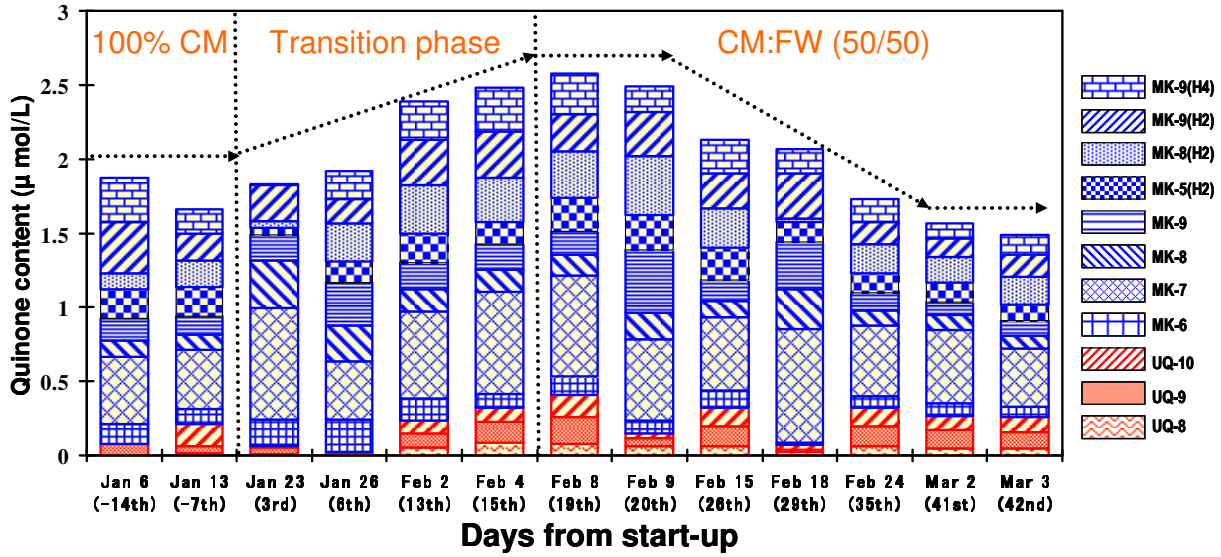

Figure 5. Quinone profile during fermentation of CM with FW

Relationship between Biogas Production and Quinone Profiles

A correlation between biogas production rate and total quinone profiles is shown in Figure 6.

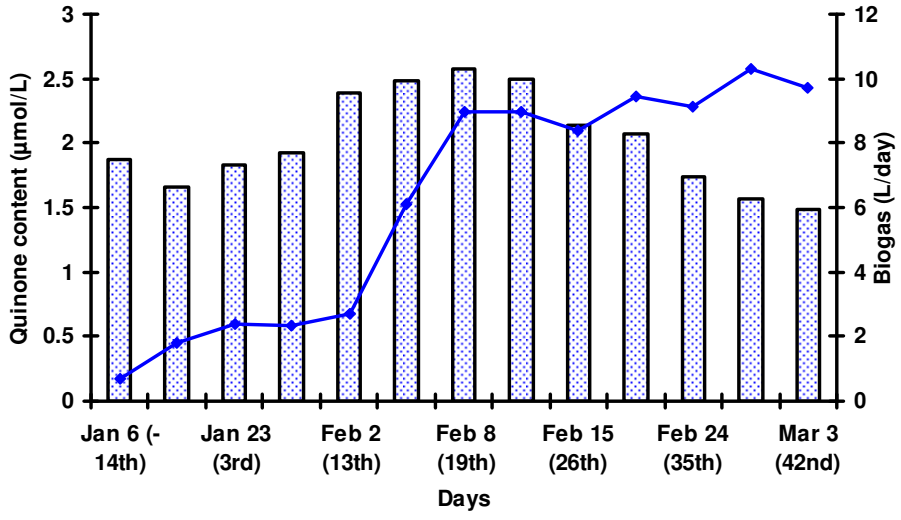

Figure 6. Relation of Quinone Amount and Biogas Production

Quinone amount was optimized, on February 8th corresponding to the ratio cow manure to food waste (50:50), at the same time with the first peak of the biogas production detected at highest values for both 2.5 µmol/l and 8.5l/day respectively. They gradually reduced together after 7 days, the quinone amount still decreasing while the biogas yield increased again and tend to reach a relative stability. This can be explained by the drop of the carbon content that correlated with the biogas production as reported by Ağdağ et al.. 2006. However, from March 2nd, quinone amount start to stabilize as the biogas production.

Conclusions

Anaerobic co-digestion process of cow manure and simulated food waste was investigated based on quinone profile using supercritical fluid extraction. Quinone analysis can be used as chemical biomarker to control the microbial community in anaerobic digestión. Quinone is a useful method to monitor the change in microbial community and overcome digester instability. Addition of FW as co-substrate in CM digestion resulted in an increase in quinone amount, volatile solids removal and biogas production. Change in quinone content was correlated to the biogas yield, therefore on the basis of these results a strong relationship between quinone and biogas production can be established.

Acknowledgements

The authors would like to thank the Nagoya Greater initiative for providing opportunity to the first author as a visiting researcher at Toyohashi University of Technology, Japan during which this reasearch was conducted.

References

Ağdağ O.N. & Sponza D.T. 2006. Co-digestion of mixed industrial sludge with municipal solid wastes in anaerobic simulated landfilling bioreactors. *Journal of Hazardous Materials.*

Arata Katayama, Koichi Fujie. Characterization of soil Microbiota with Quinone profile. *Nagoya University*, and *Toyoyhashi University of Technology.*

Aikira Hiraishi. 1999. Isoprenoid quinones as biomarkers of Microbial populations in the Environment. *Journal of Bioscience and Bioengineering*. Vol. 88, No., 5, 449-460.

Amann R. I, Ludwig W & Schleifer K. H. 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev. 59: 143–169

Collins, M.D., Jones, D., 1981. Distribution of isoprenoid quinone structure types in bacteria and their taxonomic implications. *Microbiol. Rev*. 45, 316-354.

Dean P. O' Grady, Philip H. Howard, and A. Frances Werner. 1984. Activated sludge biodegradation of 12 commercial phthalate esters. Applied and Environmental Microbiology. Vol. 49 (2).

Farrelly, V., Rainey, F.A., Stackebrandt, E. 1995. Effect of genome size and *rrn* gene copy number on PCR amplification 16S rRNA genes from a mixture of bacterial species. Appl.. Environ. Microbiol. 61, 2798–2801.

Hedrick, I.A., White D.C. 1986. Microbial respiratory quinones in the environment. *J. Microbiol. Methods* 5, 243-254.

Hong-Yong Hu, Byung-Ran Lim, Naohiro Goto, Koichi Fujie. 2001. Analytical precisión and repeatability of respiratory quinones for quantitative study of microbial community structure in environmental samples. *Journal of Microbiology Methods* 47, P. 17-24.

John R. Williams, Anthony A. Clifford. 2000. *Supercritical Fluid Methods and Protocols*. Humana Press. Totowa, New Jersey.

McCarty, Perry L. 1964. Anaerobic waste treatment fundamentals. Part 1-4, *PublicWorks.*

Callaghan, F.J., Wase, D.A.J., Thayanithy, K., Forster, C.F. 1999. Co-digestion of waste organic solids: batch studies. Bioresour. Technol. 67, 117–122.

Yan, J., Kurisu, G., Cramer, W. A. 2006. Intraprotein transfer of the quinone analogue inhibitor 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone in the cytochrome b_6f complex. *Proc Natl Acad Sci U S A*. 103: 69-74.