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XYLOSE SUBSTRATE AS THE ONLY NUTRIENT IN THE OPERATION OF MICROBIAL FUEL CELLS

Microbial fuel cells are bioelectrochemical devices converting chemical energy of organic compounds (i.e. biomass) into electrical energy by catalytic reactions of microbes under anaerobic conditions. The operation of two-chamber microbial fuel cells by *Shewanella putrefaciens* and mesophilic anaerobic sludge was studied comparatively, using xylose and glucose as solo substrates during the experiments. It was found that higher electric power was generated by the multicultural system than by the monoculture.

1. INTRODUCTION

Microbial fuel cells (MFCs) show a great promise as renewable "green" bioenergy sources [1, 2]. The MFC is a bioreactor converting chemical energy of organic compounds to electrical energy by catalytic reactions of microbes under anaerobic conditions [3–5], i.e. certain microorganisms can be used in MFCs to generate electricity. MFC in general consists of an anodic and cathodic chamber divided by a proton selective membrane. Microbes in the anodic cell oxidize substrates and generate electrons and protons in the process. Electrons are attracted by the anode and are transported to the cathode through an external circuit (wire), meanwhile protons are passed through the membrane and enter the cathode cell where they are combined with oxygen and thus, forming water.

MFCs can be operated by monocultures (i.e. by certain microorganism) or multicultures (microbial consortia). In monoculture system, *Shewanella putrefaciens* is one of the most often used strains [6–8]. It is a Gram-negative, facultative anaerobic bacterium and has the ability to reduce iron and manganese, hence it can utilize both as terminal electron acceptors in the electron transport chain. Electrons are generated during the NAD – NADH conversion and generation of H^+ [8].

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In MFCs with multicultural system hundreds of various strains work together in a symbiotic way to provide free electrons. Mesophilic anaerobic sludge obtained from biogas fermenters seems to be a suitable microbiological source for the operation of MFCs since – after an incubation period – the microorganisms present in the consortia are able to produce electric energy, as well [9]. The effect of various substrates on the power of MFC has been investigated for long time [10] in order to enhance the electric energy and to extend the stable operation time. In addition, operation of MFCs can be combined with waste water treatment since they are capable to degrade certain (waste) compounds to produce energy, too [11, 12].

The aim of this work was to compare the mono- and multicultural MFCs from a special aspect: how much the MFCs are able to metabolise (convert) and generate power using *xylose* as a solo and quite "simple" carbon source.

Logan and co-workers have already reported that multicultural systems could operate better than monoculture (*S. putrefaciens*) [13]. They used complex substrates (waste water, hydrolysates) in the experiments. In another relevant work, 12 monosaccharide substrates were tested by a multicultural system [14] and it was found, that – among others – xylose could be converted into power, though it is not the "best" substrate. Finally, metabolism of xylose was studied [15] and it was found that the conversion ("electrogenesis") itself is a multistep process: several intermediates (ethanol, acetate, propionate, lactate, formate) were formed.

In these papers, however, it was not proven that multicultural system works better than the monocultural one if only xylose is applied as a substrate. Therefore we intended to complete the studies based on the earlier findings and a series of experiments were designed. Xylose can be obtained from the hemicellulose fraction of renewable, lignocellulotic biomass (similar to glucose from starch and cellulose), therefore they are considered as potential nutrient components in "green" energy applications such as MFCs.

2. MATERIALS AND METHODS

Materials. *Shewanella putrefaciens*, the exoelectrogenic bacteria was purchased from the collection of microorganisms of University of Corvinus (Budapest, Hungary). The necessary nutrients (meat extract, peptone and agar) for the microbe were ordered from Reanal (Hungary). Potassiumdichromate, silversulphate and mercuricsulphate also from Reanal were used for COD analysis. The substrates for the experiments: glucose and xylose (technical grade) were derived from Biolab (Hungary).

Microbial fuel cells (MFCs). The two-chamber microbial fuel cells used in the work were designed and constructed in our laboratory (Fig. 1). Two MFCs with similar volumes were used in our investigation: one could be sterilized (for *S. putrefaciens*) and the other was not.

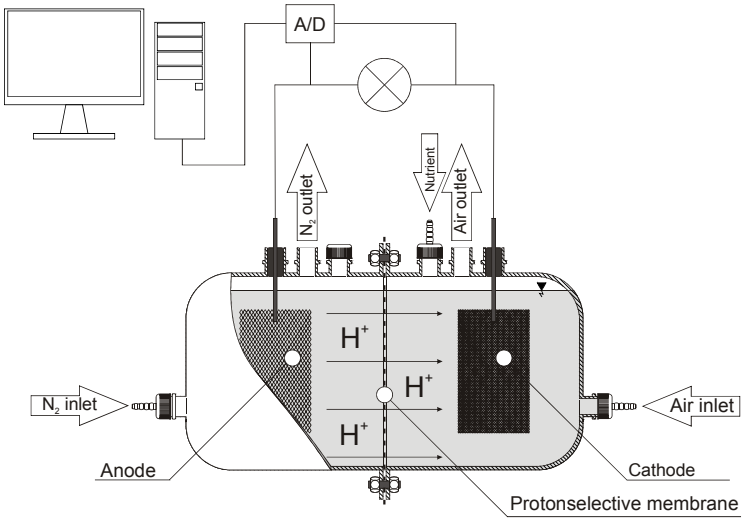

Fig. 1. Scheme of the microbial fuel cells

They had a total volume of 300 and 400 cm^3 in the anodic and cathodic chamber, respectively. The surface area of the anode and cathode located in the cells was 110 cm^2 and 49 cm^2, respectively. The anode was a carbon cloth, while the cathode was made of graphite shot by platinum. The cation-selective membrane was Nafion N115 with a 64 cm^2 surface area and 125 μm thickness. The two electrodes were connected by an external circuit, which consisted of a computerized data collecting system (National Instruments USB-6008/6009) applying LabView 8.5 software and a resistance with 10 Ω. The data were collected every 10 s in millivolts, which were shown graphically on the interface of software.

The voltage data were measured by a parallel measuring system containing a 100 MΩ resistor. Based on the voltage and resistance data, the current values were calculated, thus the current density (referred to the electrode surface area) and the electric power were possible to be provided. The cumulated electricity data were summarized by taking into account the operating time. Optimal temperature conditions are necessary for microorganisms and therefore, the device was placed in a biothermos, where the internal temperature was maintained as 37 °C. In the cathodic cell, air was introduced continuously into distilled water by a pump to ensure aerobic environment, while N_2 was sparged through the anodic cell to assure the anaerobic conditions (Fig. 2).

The MFC reactor was initially inoculated by anaerobic sludge (from a local biogas plant) in the case of the multicultural system and *S. putrefaciens* in the pure culture system. This bacteria was grown on the nutrients listed in Table 1.

After the growing period of microbes xylose was added to the systems at certain times to compare the behaviour of the two systems. Moreover glucose was added, too,

in another series of experiments, to observe similarities and differences to xylose. The ability for electric power generation in both systems was determined.

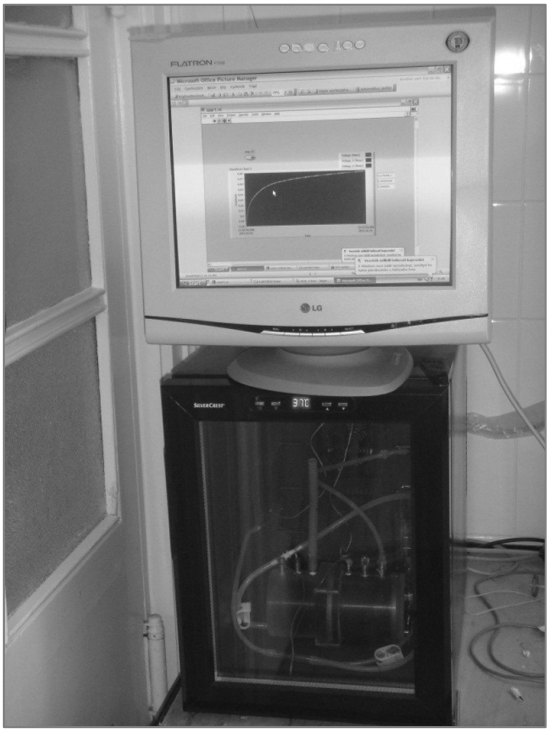

Fig. 2. Laboratory equipment used

Table 1

The applied nutrients

Nutrient	Concentration [g/100cm^3]
Meat extract	0.3
Peptone	0.5
Agar	1.5

Analysis. Samples were taken regularly from the cells and pH and total suspended solid (TSS) were determined during the experiments. In case of monoculture system xylose and glucose content were determined by measuring the reducing sugar content using the dinitro-salicylic test (DNS standard method) [16]) which is based on the formation of a chromophore between DNS product and reducing groups of the (oligo)galacturonic acid molecules. In the measurements with the multiculture system COD with bichromate was determined in the samples taken.

3. RESULTS AND DISCUSSION

3.1. MFC WITH *S. PUTREFACIENS*

Experiments were carried out to study the behaviour of MFC using *S. putrefaciens*. As can be seen in Figs. 3 and 4, in the first period (growing or adaptation period), no nutrients were added, and the voltage data were increasing up to 5.5–6 mV. Then the voltage decreased sharply and xylose/glucose was added in the 160th hour (final concentration was 4.0 g/dm^3), respectively.

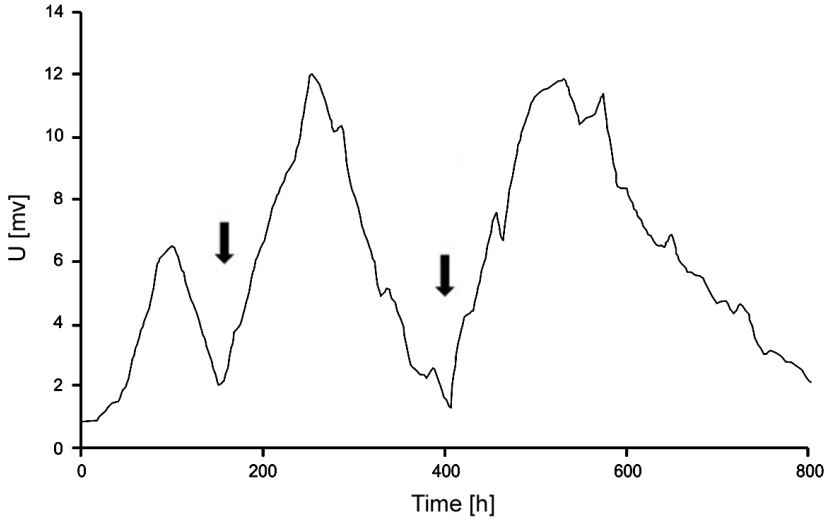

Fig. 3. The effect of "xylose only" feeding on the voltage data, *S. putrefaciens*

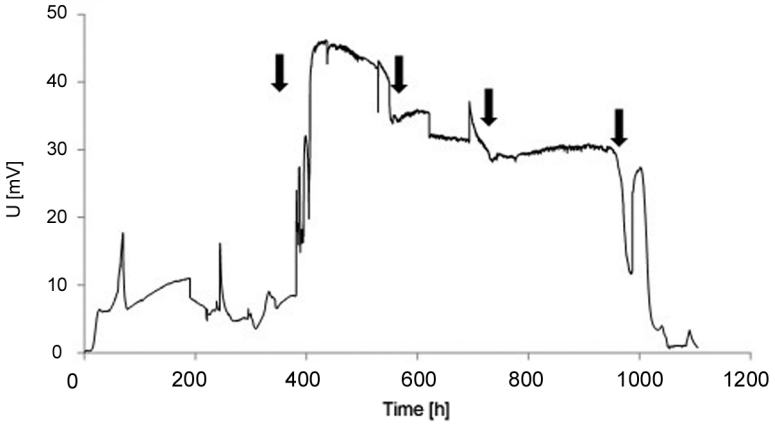

Fig. 4. Operation of the MFC with *S. putrefaciens* using glucose as substrate

In the case of xylose faster and higher response could be observed, the feeding resulted in higher power generation than in the case of glucose. Around 400 h another substrate portion was added. Using glucose, it seemed that the electric power generation was not possible to be continued, consequently the operation was stopped after about 580 h. While in the case of xylose another peak was obtained and more power was generated. During the operation less samples were taken from the monoculture system, since it was important to save the sterile conditions in the system (every sample means a risk for contamination), on one hand, and it is usually easier to follow a system with pure microorganism, on the other hand.

Table 2

Results of monoculture MFCs using xylose and glucose substrates

Time [h]	Experiment with xylose		Experiment with glucose	
	pH	TSS [g/dm^3]	pH	TSS [g/dm^3]
1	7.2	7.4	7.2	7.4
160	7.3	7.2	7.1	7.1
300	7.1	6.9	7.2	6.8
470	7.2	6.8	7.3	6.8
580	7.3	6.6	7.0	6.8

In the samples from both systems (using the two substrates), the values of pH and TSS were quite similar (Table 2). The pH was around 7.2 and the TSS was found initially as 7.4 g/dm^3 which decreased slightly. The monosaccharide content of the samples was determined, as well. It turned out that both substrates were degraded quickly by the strain, though the power was not increased proportionally. The explanation of this behaviour is given by Huang and Logan [15], where the authors described that monosaccharide degradation and power generation do not occur simultaneously but intermediate compounds (mainly short chain organic acids: e.g. acetate, propionate, lactate)) are formed, thus power is generated after a while.

Altogether 52.8 mWh/m^2 and 20.6 mWh/m^2 energies were generated by the MFC with *Shewanella putrefaciens* employing xylose and glucose as substrates, respectively, which are in the same order of magnitude values reported in the literature earlier [17]. Based on these results we can conclude that xylose seems a better substrate for MFCs from the power generation point of view.

3.2. MFC WITH MESOPHILIC ANAEROBIC SLUDGE

Based on our preliminary experiments with the mesophilic anaerobic sludge, the operational parameters of the MFC were determined. Moreover the effects of various substrates were studied by using starch, Na acetate and glucose [9]. It was found that the highest power values could be achieved when glucose was used in the system.

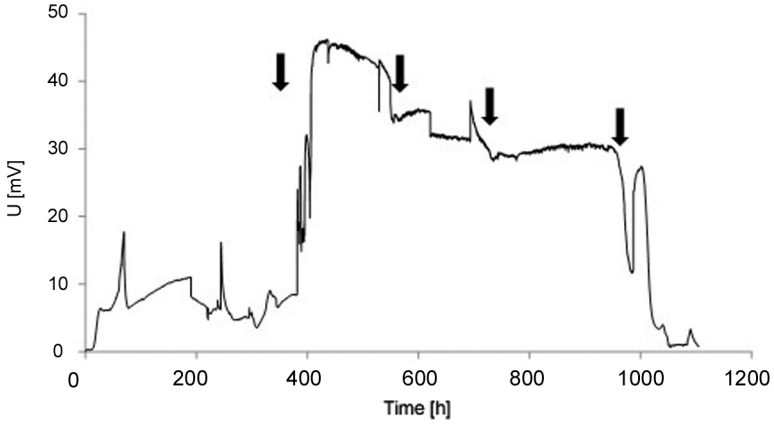

Fig. 5. The effect of xylose feeding on the voltage data, multicultural system

In this experiment, xylose was applied as substrate in the MFC, the data are shown in Fig. 5. The growing period here was similarly 300 h, the fluctuation of the voltage values was a bit greater than earlier. The first substrate addition was at around 300 h (final concentration in the anodic cell was 4 g/dm^3). Shortly, the voltage was increased significantly up to 47 mV which was followed by a slow decreasing time interval where the voltage remained still above 40 mV. Around 550 h, the power declined, therefore another substrate addition was carried out (concentration 4 g/m^3). The decreasing tendency stopped, and stagnation could be observed. The voltage data did not reach the earlier high values, but they were still above 30 mV. In the 720th h xylose substrate was added to the system again (final concentration was 4 g/dm^3) and the power started to increase slowly, during approximately 200 h. Then an unexpected and sudden decrease was observed in the 950th h of operation. Substrate was fed immediately and the voltage data increased substantially again, however the system declined quickly probably due to the accumulated exhausted sludge. Finally, after more than 1100 h operation, the experiment was terminated.

The COD, pH and TSS values determined are demonstrated in Tables 3 and 4. High COD degradation was observed during the almost 1000 h long operation and similarly the TSS was decreased. pH was varied in the range of 6.5 and 7.4, which implies the consistent operation.

For comparison, a similar and simultaneous experiment was carried out with *glucose* substrate. The experimental data are shown in Fig. 6. As can be seen in the first (growing) period, there was no substrate addition (feed). After inoculation, the adaptation of the strains took place. After approximately 100 h power was generated, 22–28 mV voltage values were recorded, which equals to 6.145 mW/m^2 power density. Afterwards, the nutrient concentrations were decreased and the power decreased, as well. Glucose was added to the system (300 h) thus 5 g/dm^3 final concentration in the anodic cell was adjusted. The electron formation was immediately lifted up and it

was growing continuously. During 180 h operation, the voltage measured was 26 mV. When voltage decreased again, the anodic cell was resupplemented by another portion of substrate (final concentration was 4 g/dm^3) which resulted in even higher power generation (38 mV – 13.13 mW/m^2) [18]. It was maintained roughly for 130 h.

Table 3

Decrease of COD using xylose substrate (multicultural system)

Time [h]	COD [mg/dm^3]		Decrease of COD [%]
	After feeding	End of period	
1–380	7500	3180	58
380–580	7675	2125	72
580–720	6620	3256	51
720–980	7750	3262	58

Table 4

TSS and pH determined (multicultural system, xylose)

Time [h]	pH	TSS [g/dm^3]
1	7.4	8.4
380	6.9	3.8
580	7.0	3.4
720	7.1	2.6
980	6.5	2.4

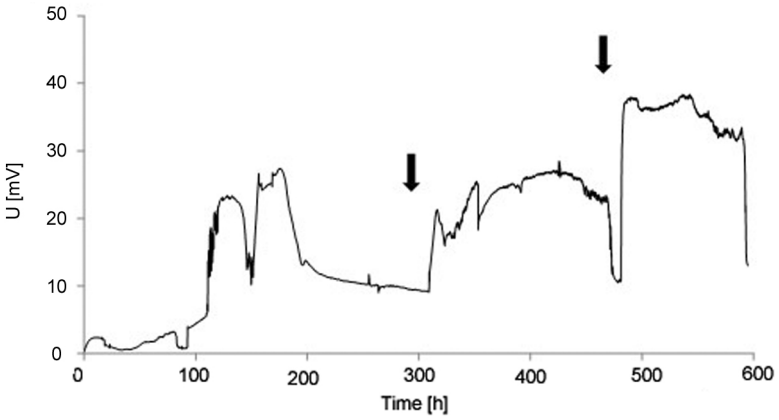

Fig. 6. Voltage data of MFC operated with sludge, with glucose feeding

The COD, pH and TSS values of the samples – for characterization of the MFC system – were determined and summarised in Tables 5 and 6. As can be seen high

COD degradation was achieved during the operation and the TSS was decreased simultaneously, while pH was not changed significantly that confirmed the reliable operation.

Table 5

COD degradation data during the experiment
by multicultural system using glucose as substrate

Time [h]	COD [mg/dm^3]		Decreasing of COD [%]
	After feeding	End of period	
1–300	8750	4080	53
300–470	7144	3125	57
470–600	4964	1200	76

Table 6

TSS and pH of the samples (multicultural system, glucose)

Time, h	1	300	470
pH	7.4	7.0	7.2
TSS, g/dm^3	7.8	5.4	3.4

3.3. COMPARISON

The achievements of the experiments in the MFCs by mono- and multicultural systems using xylose and glucose substrates were compared. Based on the experimental results, we can state now that both mono- and multicultural MFCs were able to metabolise and generate power using xylose as an only substrate. Moreover we found that the cumulated energy data (ΣP) for the *S. putrefaciens* were much lower than the multicultural systems for both substrates, thus the MFC inoculated with the anaerobic sludge worked better under the given circumstances.

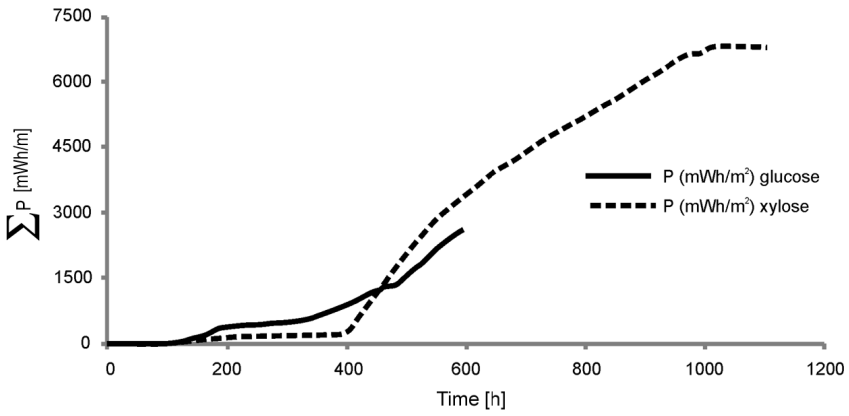

Fig. 7. Comparison: cumulated energy data for glucose and xylose feeding (sludge)

Cumulated energy values of the multicultural MFCs are presented in Fig. 7, in the case of glucose and xylose feeding. As it can be seen higher values were obtained when xylose was the substrate and longer operation time was possible to be maintained. These data suggested that xylose was probably the preferred substrate.

Table 7

Calculated parameters for the MFC systems

MFC	J [mA/m^2]	P_{anode} [mW/m^2]	P_V [mW/m^3]	$\sum P$ [mWh/m^2]
S. putrefaciens with xylose (4 g/dm^3) in MFC	43.5	0.11	3.24	52.8
S. putrefaciens with glucose (4 g/dm^3) in MFC	20.0	0.04	1.47	20.6
MFC sludge with xylose (4 g/dm^3)	418.18	19.23	528.83	6793
MFC sludge with glucose (4 g/dm^3)	345.45	13.13	361.1	2600

Summarising the results of both MFCs and substrate, in Table 7 the current density J, the power related to the anode surface area $P_{anode,}$ the volume P_V, and finally, the total cumulated energy values are given. As can be seen, all the parameters calculated were much higher for the multicultural system (using mesophilic anaerobic sludge) than for the monocultural one, moreover it is worth mentioning that xylose as a substrate resulted in higher energy values than glucose in both cases.

4. CONCLUSION

The operation of two-chamber MFCs applying *S. putrefaciens* and mesophilic anaerobic sludge was studied comparatively, using xylose (and – for comparison – glucose) as the only substrate during the experiments. Both mono- and multicultural MFCs were able to metabolise and generate power using xylose as an only substrate but multicultural MFC worked better, as the experimental results (current density, power related to the anode surface area and the volume, as well as the cumulated energy data) confirmed it.

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