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Effect of Mass Transfer on Performance of Microbial Fuel Cell

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1. Introduction

As the energy sources decrease and the climate conditions change, demand for new and clean sources of energy has increased (Hong et al., 2009; Li et al., 2010a). Fuel cells, as a high efficiency energy converting device, have attracted more and more attention recently with low/zero emission (Liu et al., 2006). Moreover, conventional sewage treatment requires high energy and capital cost so there is great interest for finding clean and sustainable energy with very low or zero emission and cost effective that is an alternative for treatment technology (Appleby, 1988; Min et al., 2005).

Microbial fuel cells (MFCs) are one kind of fuel cell and also new source of energy. In MFCs, electrons are supplied from chemical bonds with the aids of microorganisms. Then the produced electrons are transported to anode surfaces and protons are moved through proton exchange membrane or salt bridge toward cathode (Wen et al., 2009). The electron flows through an electrical external circuit while anode is connected to cathode. The flow of electron has a current (I) and power (P) is resulted. The reduction of organic substances in anode was catalyzed by the living organism in anode chamber (Chen et al., 2008; Rahimnejad et al., 2009)

Traditional MFC is consist of two separated chambers named cathode and anode ones. Oxidation of substrate by microorganisms leads to generation of electrons and protons in anaerobic anode compartment. (Rahimnejad et al., 2009). A typical biological fuel cell is shown schematically in Fig.1.

Several parameters affect on the performance of MFC, namely microbial inoculums, chemical substrates, mass transfer area, absence or existence of proton exchange materials, mechanism of electron transfer to the anode surface ,cell internal and external resistance, solution ionic strength, electrode materials and the electrode spacing (Park and Zeikus, 2000; Gil et al., 2003; Rosenbaum et al., 2007; Zhang et al., 2007; Li et al., 2010b)

Direct electron transfers from anaerobic anode chamber to anode surface had shown to take place only at very low efficiency (Park et al., 2000; Lovley, 2006). Electron transfer efficiencies in MFCs would be improved with the use of suitable electron mediators. Most MFCs use electron mediator component to improve the output of the cells. It has been reported in the literature that mediators are artificially added to anode chamber, such as Methylen blue (MB), Neutral red (NR), Thionin, Ferricyanide, Humic acid or Methyl viologen (Kim and Lee). The presence of artificial electron mediators are essential in some of MFCs to improve the performance of MFCs (Park and Zeikus, 1999; 2000). But recently,



Fig. 1. A typical MFC representing current generation with the help of microorganisms (Shukla et al., 2004)

mediators less MFCs became an interesting issue for many researchers (Kim et al., 2002; Chaudhuri and Lovley, 2003; Venkata Mohan et al., 2007; Huang et al., 2008; Venkata Mohan et al., 2008). Table 1 shows a list of MFCs were examined with or without mediators used as component along with substrate.

Microorganism	Substrate	Mediators	Reference	
Geobacter sulfurreducens	Acetate	Without mediator	(Bond and Lovley, 2003)	
Saccharomyces cerevisiae	Hydrolyzed Lactose	MB, NR	(Najafpour et al.)	
Saccharomyces cerevisiae	Glucose	NR	(RAHIMNEJAD et al.; Rahimnejad et al., 2009)	
Saccharomyces cerevisiae	Glucose	Resorufin	(Ganguli and Dunn, 2009)	
Aeromonas hydrophila	Glucose, Acetate	Without mediator	(Pham et al., 2003)	
Enterococcus faecium	Glucose	Pyocyanin	(Rabaey et al., 2005a)	
Streptococcus lactis	Glucose	Ferric Chelate complex	(Vega and Fernández, 1987)	
Proteus vulgaris	Glucose, Maltose, Galactose	Thionin	(Lee et al., 2002)	
Gluconobacter oxydans	Glucose	HNQ, Resazurin, Thioninee	(Lee et al., 2002)	
Shewanella putrefaciens	Lactate	Without mediator	(Kim et al., 2002)	
Domestic wste water	Glucose, Xylose	Humic acid	(Thygesen et al., 2009)	
Geobacter sulfurreducens	Acetate	Without mediator	(Yi et al., 2009)	
Rhodoferax ferrireducens	Glucose	Without mediator	(Chaudhuri and Lovley, 2003)	
Activated sludge	Waste water	Without mediator	(Kim et al., 2004)	
Mixed consortium	Glucose, Sucrose	Without mediator	(Rabaey et al., 2005b)	
Actinobacillus succinogenes	Glucose	NR, Thionine	(Park and Zeikus, 2002)	
Klebsiella pneumoniae	Glucose	HNQ	(Rhoads et al., 2005)	
Micrococcus luteus	Glucose	Thionine	(Choi et al., 2007)	
Shewanella oneidensis	Lactate	Anthraquinone-2,6-disulfonate (AQDS)	(Ringeisen et al., 2006)	
Escherichia coli	Glucose, Acetate	NR, 2-Hydroxy-1,4- Naphthoquinone, MB	(Bennetto, 1990; Park et al., 2000; Schröder et al., 2003; Grzebyk and Pozniak, 2005; Ieropoulos et al., 2005)	
Proteus vulgaris	Glucose, Sucrose	Thioninee	(Bennetto et al., 1985; Thurston et al., 1985; Shin et al., 2006)	
Proteus mirabilis	Glucose	Thionine	(Choi et al., 2003)	
Shewanella putrefaciens	Glucose, Lactate	Without mediator	(Kim et al., 2002)	

Table 1. Microorganisms used in MFC

Commonly oxygen as terminal electron acceptor was used in cathode compartment. Consumption of electrons and protons that are combined with oxygen, forms water at last, and end this transfer cycle. Oxidized mediators, can also accelerate reaction of forming water in cathode chamber (Heitner-Wirguin, 1996).

The objective of this chapter was to demonstrate the power production from glucose as sole electron donors in MFC. But the main purpose of this present research was to investigated the effect of mass transfer area on MFCs performance.

2. Materials and methods

2.1 Microorganism and cultivation

Saccharomyces cerevisiae PTCC 5269 was supplied by Iranian Research Organization for Science and Technology (Tehran, Iran). The microorganisms were grown at anaerobic condition in an anaerobic jar vessel. The prepared medium for seed culture consisted of glucose, yeast extract, NH₄Cl, NaH₂PO₄, MgSO₄ and MnSO₄: 10, 3, 0.2, 0.6, 0.2 and 0.05 g.l⁻¹, respectively.

The medium pH was initially adjusted to 6.5 and the inoculums were introduced into the media at ambient temperature. The inoculated cultures were incubated at 30°C. The bacteria were fully grown in a 100ml flask without any agitation for the duration of 24 hours. Substrate consumption was calculated based on determination of the remaining sugars in the culture. Growth was monitored by measuring the optical density (OD at 620_{nm}). Substrate consumption was calculated based on determination of the remained sugars in the culture according to Sadasivam and Manickam(Sadasivam and Manickam, 2005).

2.2 Chemical and analysis

All chemicals and reagents used for the experiments were analytical grades and supplied by Merck (Darmstadt, Germany). The pH meter, HANA 211(Romania) model glass-electrode was employed to measure pH values of the aqueous phase. The initial pH of the working solution was adjusted by addition of diluted HNO3 or 0.1M NaOH solutions. Dinitrosalicylic acid [3, $5(NO_2)_2C_6H_2$ -2OH-COONa.H₂O] (DNS) method was developed to detect and measure substrate consumption using colorimetric method. Before analysis, liquid samples were filtered by a 0.45 µm syringe membrane (Sartorius Minisart).

Scan Electron Microscope (SEM): The anode electrode before and at the end of the experiment was examined by a Scanning Electronic Microscope (SEM) (Phillips XL30, Holland). Finally, images of the samples were taken under SEM at magnifications of 5000. SEM images were used to demonstrate the physical characteristics of the electrode surface and to examine the growth of yeast on the anode surface.

2.3 MFC

Different kinds of MFCs were made up to investigation of mass transfer area on performance of MFC. All MFCs fabricated from Plexiglas material were used as MFCs in laboratory scale. The volume of each chamber (anode and cathode chambers) was 800 ml with a working volume of 615 ml. The sample port was provided for the anode chamber, wire point input and inlet port. The selected electrodes in MFC were graphite plates, size of 40×70×1.2mm. Proton exchange membrane (PEM; NAFION 117, Sigma–Aldrich) was used to separate two compartments. Proton exchange membrane, nafion, was subjected to a course of pretreatment to take off any impurities that was boiling for 1h in 3% H₂O₂, washed with deionized water, 0.5 M H₂SO₄, and finally washed with deionized water. In order to maintain membrane for good conductivity, the anode and cathode compartments were filled with deionized water when the MFC was not in use. Neutral red and potassium

permanganate were also supplied by Merck Company (Darmstadt, Germany) as mediators and oxidizer agent in continues mode, respectively. The schematic diagram, photographic images and auxiliary equipments of the fabricated MFC cell in batch and continuous systems are shown in Fig. 2. In continuous operation, the MFC was continuously fed with the prepared media in an up-flow mode using an adjustable peristaltic pump (THOMAS, Germany).



Fig. 2. Schematic diagram of cubic two chamber MFC in batch (a) and continues (b) mode

2.4 Analytical method

Two protocols, polarity and cyclic voltammetry techniques, were adopted to analyze experimental data in terms of voltage and current density.

2.4.1 Polarity curve

Polarization curves were obtained using an adjustable external resistance. Power and current were calculated based on following equations:

$$P=I\times E \tag{1}$$

$$I = (E/R_{ext}) \tag{2}$$

where P is generated power and E measured cell voltage; Rext denotes external resistance and I indicates produced current. The online recorded produced current and power were normalized by the surface area of the used membrane. Analog digital data acquisition was fabricated to record data point in every 4 min. Measurements were carried out at variable resistances which were imposed to the MFC. The current in the MFC was automatically calculated and recorded dividing the obtained voltage by the specified resistance. Then, the system provides power calculation by multiplication of voltage and current. The provisions were provided for online observation of polarization curve showing the variation of power density and MFC voltage with respect to current. The online system had the ability to operate automatically or manually. While it operates in auto-mode, the assembled relays were able to regulate automatically the resistances. Voltage of MFC was amplified and then data were transmitted to a microcontroller by an accurate analog to digital converter. The microcontroller was also able to send the primary data to a computer by serial connection. In addition, a special function of MATLAB software (7.4, 2007a) was used to store and display synchronically the obtained data. The power, current and voltage were automatically recorded by the computer connected to the system.

Columbic efficiency can be calculated by division of total coulombs obtained from the cell and theoretical amount of coulombs that can be produced from glucose (Equation 3):

$$CE = (C_p/C_T) \times 100 \tag{3}$$

Total coulombs are obtained by integrating the current variation over time (C_p), where C_T is the theoretical amount of coulombs that can be produced from carbon source, calculated as follows:

$$C_T = (FbSV.M^{-1}) \tag{4}$$

For continuous flow through the system, CE can be calculated on the basis of generated current at steady state conditions as follows (Logan et al., 2006):

$$CE = MI/Fbq\Delta S \tag{5}$$

In equation (4), *F* is Faraday's constant , b the number of moles of electrons produced per mole of substrate (24 mol of electrons were produced in glucose oxidation in anaerobic anode chamber), *S* the substrate concentration, *q* flow rate of substrate and *M* the molecular weight of used substrate (M= 180.155 g.mol⁻¹) (Allen and Bennetto, 1993; Oh and Logan, 2006).

In batch mode, polarization curves were obtained at steady state condition by setting an adjustable resistance in data logger. When the MFC was operated in continuous mode, the concentration of glucose in the feed tank solution was kept constant at 30 g.l-1. Several hydraulic retention times (HRT) were examined in continuous operation. The HRT was measured from the volume of medium and the inward flow rate to the anode compartment of MFC.

2.4.2 Cyclic Voltammetry (CV)

Beside the polarity curve, cyclic Voltammeter (IVUM soft, Ivium Technology, Netherland) was also used to analyze for testing oxidation and reduction of organic materials. The potential range of -400 mV to 1000 mV was applied. The working electrode and sense

electrode were joined together to measure oxidation and reduction peaks. Carbon paper (NARA, Guro-GU, Seoul, Korea) was used as the working electrode and Platinum (Platinum, gauze, 100 mesh, 99.9% meta basis, Sigma Aldrich) as the counter electrode. Also, Ag/AgCl (Ag/AgCl, sat KCl, Sensortechnik Meinsberg, Germany) electrode was utilized as reference electrode. Voltage rate of 50 mV.S⁻¹ was chosen as scan rate in CV analysis.

3. Result and discussion

Microorganism can be used in MFCs to catalyze the conversion of organic matter into electricity. The performance of the MFC was evaluated by the polarization curve and power density. The main goal of research to work on MFC is to increase output power and receive maximum generated current under optimum potential conditions.

Polarization behavior of the fabricated cell was recorded for several external resistances to determine maximum power generation. Polarization curve and power density vs. current density of the cell after 12 hours incubation and also reaching to steady state (SS) condition are presented in Fig. 3. The maximum produced power without any electron shuttle in anode was 4 mW.m⁻². The produced power and current were very low to use in a small device and it must be improved.

Mediators are normally used to enhance the performance of MFCs (Najafpour et al.). Mediators are artificial compounds or produced by the microorganism itself. Some microorganisms produce nanowires to transmit electrons directly without using any mediator but other organisms need to add artificial electron shuttle into anode chamber (Mathuriya and Sharma, 2009). Yeast cannot transfer the produced electrons to the anode surface without addition of mediators. In orther to improve the power density and also current density several mediators with several concentrations were selected to enhance the power generation and current in the fabricated MFC. The maximum power, maximum current and also the obtained OCV at the best concentration of each mediator are summarized in Table 2. The data indicated that the mediators were essential when yeast was used as active biocatalyst in the MFC. Also this table indicated NR with concentration of 200 µmol.l⁻¹ had the best ability for transferring the generated electrons in the anode chamber to the anode surface. The indicated concentration of NR in anaerobic anode compartment increased the produced power was 46 times more than the case without mediators in the MFC.

Type of mediators	Optimum concentration (µ mol.l ⁻¹)	P _{max} (mW.m ⁻²)	I _{max} in P _{max} (mA.m ⁻²)	OCV at SS condition (mV)
Without mediators		0.8	11	280
Ferric chelate	400	7.3	67	285
Thionine	500	12	79	460
NR	200	37	151	505
MB	300	8.3	71	410

Table 2. Optimum condition obtained from this study at several concentrations of mediators



Fig. 3. Generated power density (a) and voltage (b) as function of current density at start up, 10 hours after incubation and at steady state condition

In order to obtain the best oxidizer in cathode compartment, several oxidizers were analyzed. Table 3 summarized the optimum conditions obtained for distilled water, potassium ferricyanide and potassium permanganate. The maximum power, current and OCV was obtained with potassium permanganate.

Type of Oxidizer	Optimum concentration (µ mol.l ⁻¹)	P _{max} (mW.m ⁻²)	I _{max} in P _{max} (mA.m ⁻²)	OCV at SS condition (mV)
Distillated water		7.6	68	404
H_2O_2		41	155	610
Potassium ferricyanide	200	49	177	508
Potassium Permanganate	300	110	380	860

Table 3. Optimum conditions obtained from several oxidizers

Glucose consumption and cell growth with respect to incubation time at 200µmol.l⁻¹ of NR as electron mediators are presented in Fig. 4. Figure 4 demonstrated that *S. cerevisiae* had the good possibility for consumption of organic substrate at anaerobic condition and produce bioelectricity.

The aim of this research was to found optimum effect of mass transfer area on production of power in the fabricated MFC. Figure 5 shows the effect of mass transfer area on performance



Fig. 4. Cell growth profiles and glucose consumption by S. cerevisiae



Fig. 5. Effect of mass transfer area on performance of MFC.

of MFC. Three different mass transfer area (3.14, 9and 16 cm²) were experimented and the results in polarization curve presented in Fig. 5 a and b. Membrane in MFC allows the generated hydrogen ions in the anode chamber pass through the membrane and then to be transferred to cathode chamber (Rabaey et al., 2005a; Cheng et al., 2006; Venkata Mohan et al., 2007; Aelterman et al., 2008). The obtained result shows the maximum current and power were obtained at Nafion area of 16 cm². The maximum power and current generated were 152 mW.m⁻² and 772 mA.m⁻², respectively.

Figure 6 depicts an OCV recorded by online data acquisition system connected to the MFC for duration of 72 hours. At the starting point for the experimental run, the voltage was less than 250mV and then the voltage gradually increased. After 28 hours of operation, the OCV reached to a maximum and stable value of 8mV. The OCV was quite stable for the entire operation, duration of 72 hours.



Fig. 6. Stability of OCV.OCV recorded by online data acquisition system connected to the MFC for duration of 72 hours

There are several disadvantages of batch operation for the purpose of power generation in MFCs. The nutrients available in the working volume become depleted in batch mode. The substrate depletion in batch MFCs results in a decrease in bioelectricity production with respect to time. This problem is solved in continuous MFCs that are more suitable than batch systems for practical applications (Rabaey et al., 2005c). The advantages of continuous culture are that the cell density, substrate and product concentrations remain constant while the culture is diluted with fresh media. The fresh media is sterilized or filtered and there are no cells in the inlet stream.

The batch operation was switched over to continuous operation mode by constantly injection of the prepared substrate to the anode compartment. The other factors were kept constant based on optimum conditions determined from the batch operation. For the MFC operated under continuous condition, substrate with initial glucose concentration of 30 g.l⁻¹

was continuously injected from feed tank to the anode chamber using a peristaltic pump. Four different HRT were examined in this research to determine the optimum HRT for maximum power and current density. The polarization curve at each HRT at steady state condition was recorded with online data acquisition system and the obtained data are presented in table 4. The optimum HRT was 6.7 h with the maximum generated power density of 274 mW.m⁻².

HRT	P _{max}	I max in Pmax	OCV at SS condition
(h)	(mW.m ⁻²)	(mA.m ⁻²)	(mV)
16	7 161	420	801
12.34	182	600	803
6.66	274	850	960
3.64	203	614	975

There is the contraction of production of post of and callenged in the fight of the second of the se	Table 4. Effect of a	different HRT on	production of r	power and current	in fabricated MFC
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The growth kinetics and kinetic constants were determined for continuous operation of the fabricated MFC. The growth rate was controlled and the biomass concentration was kept constant in continuous system through replacing the old culture by fresh media. The material balance for cells in a continuous culture is defined by equation 5 (Bailey and Ollis, 1976):

$$F.x_i - F.x + V.r_x = V.\frac{dx}{dt}$$
(5)

where, F is volumetric flow rate of feed and effluent liquid streams, V is volume of liquid in system, r_x is the rate of cell growth, xi represents the component i molar concentration in feed stream and x is the component i molar concentration in the reaction mixture and in the effluent stream. The rate of formation of a product is easily evaluated at steady-state condition for inlet and outlet concentrations. The dilution rate, D, is defined as D=V/F which characterizes the inverse retention time. The dilution rate is equal to the number of fermentation vessel volumes that pass through the vessel per unit time. D is the reciprocal of the mean residence time(Najafpour, 2007).

At steady-state condition, there is no accumulation. Therefore, the material balance is reduced to:

$$F.x_i - F.x + V.r_x = 0 \rightarrow V/_F = \frac{(x - x_0)}{r_x}$$
 (6)

When feed is steriled, there is no cell entering the bioreactor, which means $x_0=0$. Using the Monod equation for the specific growth rate in equation 6, the rate may be simplified and reduced to following equation:

$$r_x = \frac{dx}{dt} = \mu x = \frac{(\mu_{max} \cdot S \cdot x)}{K_s + S}$$
(7)

HRT (h)	16	12.34	6.66	3.64
X (g.l-1)	1.94	1.74	1.728	1.5
S(g.1-1)	6.95	9.13	12.86	22.8

Table 5. Biomass and substrate concentration in outlet of MFC at different HRT



Fig. 7. Effect of active biofilm on anode surface with CV analysis. (a) absence of biofilm ,(b) after formation of biofilm with out mediators and (c) after formation of biofilm with 200 μ mol.l⁻¹ NR as electron mediators .scan rate was 0.01 V.S⁻¹

Biomass and substrate concentration in outlet stream of MFC at different HRT are shown in Table 4. To evaluate kinetic parameters, the double reciprocal method was used for linearization. The terms μ_{max} and K_s were recovered from a linear fit of the experimental data by Plotting 1/D versus 1/S. The values obtained for μ_{max} and K_s were 0.715 h and 59.74 g/l, respectively. Then, the kinetic model is defined as follows:

$$r_x = \frac{(0.715 \, S. \, x)}{59.74 + S} \tag{8}$$

In the next stage, anode electrode with attached microorganisms was analyzed with CV in. The system was analyzed in anaerobic anode chamber. Before formation of active biofilm on anode surface, oxidation and reduction peak was not observed in CV test (Fig. 7a). Current-potential curves by scanning the potential from negative to positive potential after formation of active biofilm are shown in Fig. 7b. Two oxidation and one reduction peak was obtained with CV test. One peak was obtained in forward scan from -400 to 1000 mV and one oxidation and reduction peak was obtained in reverse scan rate from 1000 to -400 mV. The similar result by alcohol as electron donors in anode chamber was reported(Kim et al., 2007). The first peak was observed in forward scan rate between -0.087 to 1.6 V. Also 200 μ mol.l⁻¹ NR was added to anode chamber and then this system was examined with CV (Fig. 7 c)

Graphite was used as electrode in the MFC fabricated cells. The normal photographic image of the used electrode before employing in the MFC as anode compartment is shown in Fig. 8a. Scanning electronic microscopy technique has been applied to provide surface criteria and morphological information of the anode surface. The surface images of the graphite plate electrode were successfully obtained by SEM. The image from the surface of graphite electrode before and after experimental run was taken. The sample specimen size was 1cm×1cm for SEM analysis. Fig. 8b and 8c show the outer surface of the graphite electrode prior and after use in the MFC, respectively. These obtained images demonstrated that microorganisms were grown on the graphite surface as attached biofilm. Some clusters of microorganism growth were observed in several places on the anode surface.



Fig. 8. Photography image (a) and SEM images from anode electrode surface before (b) and after (c) using in anode compartment

Yeast as biocatalyst in the MFC consumed glucose as carbon source in the anode chamber and the produced electrons and protons. In this research, glucose was used as fuel for the MFC. The anodic and catholic reactions are taken place at the anode and cathode as summarized below:

$$C_6H_{12}O_6 + 6H_2O \longrightarrow 6CO_2 + 24 e^- + 24H^+$$
 (5)

$$6O2 + 24 e^{-} + 24H^{+} \longrightarrow 12H_2O$$
 (6)

24 mol electrons and protons are generated by oxidation of one mole of glucose in an anaerobic condition. To determine CE (Columbic Efficiency), 1 K Ω resistance was set at external circuit for 25 h and the produced current was measured. The average obtained current was 105.85 mA.m⁻². In this study, CE was calculated using equations 3 and 4. CE was 26% at optimum concentration of NR as mediator. CE at continues mode was around 13 percent and this efficiency is considered as very low efficiency. The similar results with xylose in fed-batch and continuous operations were also reported (Huang and Logan, 2008b; a). This may be due to the breakdown of sugars by microorganisms resulting in production of some intermediate products such as acetate, butyrate, and propionate, which can play a significant role in decrease of CE.

4. Chapter conclusion

MFC produce current through the action of bacteria that can pass electrons to an anode, the negative electrode of a fuel cell. The electrons flow from the anode through a wire to a cathode The idea of making electricity using biological fuel cell may not be new in theory, certainly as a practical method of energy production it is quite new. Some of MFCs don't need mediators for transfer electrons but some of others need mediators in anode chamber for transfer electrons to anode surface.

Bioelectricity production from pure glucose by *S cerevisiae* in dual chambered MFC was successfully carried out in batch and continuous modes. Potassium permanganate was used as oxidizing agent in cathode chamber to enhance the voltage. NR as electron mediator with low concentration (200 µmol.l-1) was selected as electron mediator in anode side. The highest obtained voltage was around 900 mV in batch system and it was stable for duration time of 72 h. The mass transfer area is one of the most critical parameter on MFCs performances.

5. Acknowledgments

The authors wish to acknowledge Biotechnology Research Center, Noshirvani University of Technology, Babol, Iran for the facilities provided to accomplish the present research.

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Mass Transfer in Chemical Engineering Processes Edited by Dr. Jozef MarkoÅj

ISBN 978-953-307-619-5 Hard cover, 306 pages **Publisher** InTech **Published online** 04, November, 2011 **Published in print edition** November, 2011

This book offers several solutions or approaches in solving mass transfer problems for different practical chemical engineering applications: measurements of the diffusion coefficients, estimation of the mass transfer coefficients, mass transfer limitation in separation processes like drying, extractions, absorption, membrane processes, mass transfer in the microbial fuel cell design, and problems of the mass transfer coupled with the heterogeneous combustion. I believe this book can provide its readers with interesting ideas and inspirations or direct solutions of their particular problems.

How to reference

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Mostafa Rahimnejad, Ghasem Najafpour and Ali Asghar Ghoreyshi (2011). Effect of Mass Transfer on Performance of Microbial Fuel Cell, Mass Transfer in Chemical Engineering Processes, Dr. Jozef MarkoÅ_i (Ed.), ISBN: 978-953-307-619-5, InTech, Available from: http://www.intechopen.com/books/mass-transfer-in-chemical-engineering-processes/effect-of-mass-transfer-on-performance-of-microbial-fuel-cell



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