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Électrolyse chimique et microbienne de déchets agro-industriels pour la production de composés à haute valeur ajoutée

PAR

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DÉDICACE

I dedicate this thesis to:

My beloved wife,

Nazanin

and

All scientists who perform the research for the improvement of human life

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Avant-propos

L'objectif principal de cette étude consiste à étudier les performances de formation de produits à valeur ajoutée par électro-fermentation et conversion électrochimique de déchets nutritifs. Cette thèse se compose de cinq chapitres.

Le premier chapitre de cette thèse comprend une synthèse des travaux, les objectifs de recherche, la problématique de recherche, la méthodologie adoptée, les principaux résultats obtenus et enfin une conclusion et des recommandations.

Le deuxième chapitre de cette thèse comprend une revue de littérature portant sur les différents aspects de l'électro-fermentation de déchets nutritifs en vue de la production de produits à valeur ajoutée. En effet, cette partie du travail a fait l'objet d'un article publié dans le (*Journal of Environmental Chemical Engineering ; Ali Khosravanipour Mostafazadeh, Patrick Drogui, Satinder Kaur Brar, R.D. Tyagi, Yann Le Bihan Gerardo Buelna, 5: 940-954 (2017),* **Microbial Electrosynthesis of Solvents and Alcoholic Biofuels from Nutrient Waste: A Review.**

Le troisième chapitre a pour objectif l'étude de la production du butanol (et autres sous-produits comme les acides organiques) à partir du glucose par les techniques d'électro-fermentation. Les résultats de ces travaux, portant particulièrement sur l'optimisation et l'étude des mécanismes sont été publiés dans le *Journal of Energy Conversion and Management, 130: 165-175 (2016): Ali Khosravanipour Mostafazadeh, Patrick Drogui, Satinder Kaur Brar, R.D. Tyagi, Yann Le Bihan, Gerardo Buelna,* **Enhancement of biobutanol production by electromicrobial glucose conversion in a dual chamber fermentation cell using *C. pasteurianum.***

Le quatrième chapitre concerne l'étude de la production de 1,3 propanediol (et sous-produits tels que les acides organiques) par électrosynthèse microbienne en utilisant du glycérol pur et brut. Les résultats de cette étude ont été soumis au Journal « Biomass conversion and biorefinery», *Ali Khosravanipour Mostafazadeh, Patrick Drogui, Satinder Kaur Brar, R.D. Tyagi, Yann Le Bihan, Gerardo Buelna,* **Electro-bioreactor concept for enhanced 1,3-propanediol by using electroactive microorganism and glycerol as a sole carbon source.**

Finalement, le cinquième chapitre porte sur la production de composés à valeur ajoutée tels que l'hydroxyacétone (acétol), le dihydroxyacétone et les acides organiques par conversion électrocatalytique du glycérol. Les résultats de cette étude ont été soumis au Journal «Current Analysis on Energy and Environmental Sciences», *Ali Khosravanipour Mostafazadeh, Patrick Drogui, Satinder Kaur Brar, R.D. Tyagi, Yann Le Bihan, Gerardo Buelna, Yessika Padilla,* **An insight into electro-catalytic reactor for high value-added production from crude glycerol: optimization, product distribution and reaction pathways identifications.**

RÉSUMÉ

Premier article

L'utilisation des ressources renouvelables pour produire des produits chimiques et des carburants est de nos jours de plus en plus sollicitée. Les matières organiques issues des déchets agro-industriels peuvent être utilisées comme matière première pour la production de biocarburants et de produits biochimiques. Cette revue de littérature présente l'électrosynthèse microbienne (ESM) en tant que technologie émergente pour la production de solvants et d'alcools à partir de déchets organiques. L'utilisation d'un système assisté par l'électricité dans le métabolisme des microorganismes anaérobies a montré une amélioration de la production des solvants par rapport aux méthodes de fermentation classiques. À la lumière des intérêts actuels, les électrotrophes sont des souches microbiennes qui peuvent accepter un électron provenant de la cathode pour ensuite réduire les matières organiques (sources de carbone) en des produits biochimiques intéressants. En effet, le glucose, le glycérol et d'autres composés organiques pourraient être convertis en bio-solvants à valeur ajoutée notamment l'éthanol, le butanol, l'acétone et le propanediol. Par ailleurs, cette revue aborde la synthèse microbienne de produits chimiques, en particulier les solvants et les alcools, grâce à la réduction des substrats multi-carbone. Elle met également en évidence les avantages de l'ESM.

Deuxième article

Un ensemble d'expériences ont été réalisées pour étudier la production de biobutanol en tant que nouveau biocarburant. L'objectif de ce travail était de comprendre le mécanisme et le taux de production du biobutanol par bioélectrosynthèse (BES) en utilisant le glucose comme substrat. Quatre facteurs principaux, à savoir le matériau des électrodes (la surface de l'électrode sur le volume du réacteur, S/V = 0.083 cm^2/mL), la concentration du substrat, la température de fonctionnement et la tension appliquée ont été étudiés en mode discontinu pour fixer les conditions optimales de production maximale de butanol par *C. pasteurianum*. Le milieu standard P2 modifié (MP2) et le milieu standard minimal (MMS) ont été utilisés comme milieu de fermentation en mode de fonctionnement batch (par lots). L'optimisation statistique à l'aide de la méthodologie de plan centrale composite (CCD) a été utilisée pour maximiser la

production de butanol dans la gamme expérimentale. La production maximale de butanol à 13,3 g/L (comparativement à la valeur enregistrée (10.2 g/L) lors de l'essai contrôle) a été obtenue en appliquant 1,32 V, ce qui prouve la pertinence de cette procédure. Aussi, la production du butanol pouvait être améliorée de façon remarquable par des microorganismes électroactifs dans la chambre cathodique et ce, dans les conditions optimales d'utilisation de MMS.

Troisième article

En ce qui concerne ces travaux, une concentration de 7,42 g/L (comparativement à la valeur enregistrée (4.6 g/L) lors de l'essai contrôle) de 1,3-propanediol (1,3-PD) a été produite en utilisant EMS et *C. pasteurianum* dans un réacteur de type H en utilisant du glycérol pur et brut et ce, dans les conditions optimales suivantes : 1,6 V de tension appliquée, 33,9 ^{o}C de température et 41,3 g/L de concentration en substrat (rapport C/N de 36,9 g/g). Pour ce faire, la méthodologie de surface de réponse (MSR) a été appliquée en vue d'optimiser les conditions de fonctionnement. Un biofilm stable, généré à la surface de la cathode en feutre de graphite, contribuait à l'augmentation des voies réactionnelles conduisant à la production de 1,3-PD. De plus, l'amélioration de la production de 1,3-PD a été démontrée avec succès dans un système d'électro-bioréacteur et ce, en tenant compte de l'équilibre d'équivalent d'électrons et des calculs NADH.

Quatrième article

La conversion électrochimique du glycérol pur et brut en produits à valeur ajoutée a été étudiée. La génération maximale de produits non-acides (dihydroxyacétone, acétol et glycidol) et d'acides organiques (acide acétique, acide lactique et acide formique) ont été optimisées par la méthode de surface de réponse (MSR). Selon la chronoampérométrie et la chronopotentiométrie, l'électrosynthèse microbienne (ESM) opérée à courant constant a plus d'efficacité que lorsque la tension est maintenue constante. Les variables considérées étaient : le pH de la solution, le type de catalyseur (électrodes de Pt, Pt/Ti ou Pt noir, la surface de l'électrode sur le volume du réacteur, S/V = 0.13 cm^2/mL), l'intensité du courant, la durée de fonctionnement et la concentration de glycérol dans le réacteur électrocatalytique discontinu.

Les concentrations et le type de produits générés ainsi que le mécanisme réactionnel ont également été étudiés. En outre, les résultats ont montré que, dans des milieux très acides (pH autour de 1.4), une production élevée de produits non-acides a été obtenue en utilisant une électrode de Pt avec un rendement de 72% d'acétol et en imposant une intensité de courant de 0,31 A pendant maximum 12 heures de fonctionnement.

ABSTRACT

First article

Using renewable resources to produce chemicals and fuels is increasing. Organic materials from wastes and carbon dioxide are renewable and serve as alternative sources for the production of biofuels and biochemicals. This review provides a survey of solvents and alcohols production from organic matter by microbial electrosynthesis (MES) as a new emerging technology. Using electricity-assisted system in anaerobic microorganisms metabolism (biocatalysts) showed improvement in solvent production compared to conventional fermentation methods. Electrotrophs, the kind of microbial strains which can accept the electron from the cathode to reduce organic carbon source materials to valuable biochemicals is of interest nowadays. Glucose, glycerol, and other organic compounds could be converted into high value-added bio-solvents, such as ethanol, butanol, acetone, and propanediol. This review addresses electricity-driven microbial synthesis of chemicals especially solvents and alcohols by reduction of multi-carbon substrates considering the characteristics and advantages of MES.

Second article

A set of experiments have been performed to investigate the production of biobutanol as a novel applicable biofuel in a bioelectrolysis cell (BEC). The objective of this work was to understand the mechanism and production rate of the biobutanol by bioelectrosynthesis (BES) using glucose as a substrate. Four main factors, such as electrode materials, substrate concentration, operating temperature, and poised applied voltage were investigated in batch mode to achieve optimum condition for producing maximum butanol by *C. pasteurianum* in BEC. Standard modified P2 medium (MP2) and standard minimal medium (SMM) were used as fermentation media in batch operation mode. Numerical optimization using a central composite design (CCD) method has been used to maximize the butanol production within the experimental range. The maximum butanol production 13.31 g/L was obtained by applying 1.32 V indicating the suitability of this procedure. The results showed that by applying optimum conditions in SMM, the butanol could be enhanced remarkably by electroactive microorganisms in the cathode chamber.

Third article

We also produced 7.42 g/L of 1,3-propanediol (1,3-PD) by using EMS and *C. pasteurianum* in batch H-type reactor by using pure and crude glycerol under the following optimum conditions: 1.6 V of applied voltage, 33.9 ^{o}C of temperature, and 41.3 g/L of substrate concentration (C/N ratio of 36.9 g/g). Response surface methodology (RSM) was applied to optimize operating conditions. We observed that by generating stable biofilm on graphite felt cathode, the reaction pathways go to increase in 1,3-PD production. Moreover, by electron equivalent balance and NADH calculations, we demonstrate that the 1,3-PD was enhanced in an electro-bioreactor system successfully.

Fourth article

In this study, the electrochemical conversion of pure and crude glycerol into value-added products have been investigated. The maximum non-acidic (dihydroxyacetone/hydroxyacetone or acetol/glycidol) and organic acids (acetic acid, lactic acid, formic acid) formations were optimized using response surface methodology (RSM) method in terms of electrolyte solution (pH), catalyst (electrode) type, current intensity, duration of operation, and glycerol concentration in a batch electro-catalytic reactor using platinum (Pt), platinized titanium (Pt/Ti), and black Pt. Products concentrations and distributions, and reaction mechanism and pathway have also been investigated. The results showed that under strong acidic conditions, the highest chemical production was achieved using Pt electrode, and under the optimized conditions, high acetol yield of 72% at 0.31 A during 12 hours of operation was observed.

SOMMAIRE RÉCAPITULATIF

De nos jours, l'utilisation des ressources renouvelables pour la production de composés chimiques et de carburants est en augmentation. La synthèse de produits chimiques et de carburant à partir des déchets agro-industriels dans les cellules d'électrolyse microbienne (CEM) peut être considérée comme une solution à certaines problématiques environnementales et énergétiques. Cependant, la CEM n'a pas encore été commercialisée. Les matières organiques provenant des déchets agro-industriels sont renouvelables et pourraient être considérées comme une alternative à la production de biocarburants et de produits biochimiques. Les déchets agro-industriels chargés en nutriments tels que le glycérol brut sont réputés être des sources d'énergie qui peuvent jouer un rôle majeur dans les énergies renouvelables. L'utilisation de l'électricité dans le métabolisme des microorganismes (biocatalyseurs) a montré une amélioration de la production de solvants par rapport aux méthodes de fermentation classique. Les électrotrophes sont des souches microbiennes pouvant accepter l'électron de la cathode et convertir la source de carbone organique en produits biochimiques à valeur ajoutée. Ce procédé mettant en jeu ces microorganismes électrotrophes suscite un vif intérêt. Le glucose, le glycérol et d'autres composés organiques pourraient être transformés en bio-solvants à haute valeur ajoutée tels que le butanol et le propanediol. Les performances de l'électrosynthèse microbienne (ESM) sont influencées par des paramètres importants tels que la physiologie microbienne, le matériau d'électrode, le type d'électrolyte, le type de substrat et la tension appliquée. Le mécanisme d'interaction entre les microorganismes électroactifs et les électrodes, via le transfert d'électrons extracellulaires, en présence d'excès d'électrons dans l'électro-bioréacteur est l'aspect principal de cette technologie.

Étant donné que la fermentation microbienne des alcools, des solvants ou des biocarburants présente divers inconvénients notamment la faible productivité dont les coûts de récupération s'avèrent élevés, le procédé assisté par l'électricité (procédé ESM) serait une alternative permettant de surmonter les problèmes pré-mentionnés. Or, l'évaluation technico-économique est également requise pour l'ESM car elle est réalisée pour la fermentation conventionnelle de biohydrogène et de produits biochimiques. Les matériaux d'électrodes ayant une forte capacité de transfert d'électron sont souvent recommandés dans les procédés ESM. Les électrodes à

base de carbone rentrent dans cette catégorie d'électrode. Elles présentent certains avantages, à savoir une bonne stabilité et un coût relativement faible. Le réacteur ESM requière des autoclaves, des électrodes et des séparateurs (membranes) qui ne doivent pas être négligés lors de la conception du bioréacteur. En résumé, certaines contraintes pourraient être surmontées grâce à l'ESM, tout en orientant le processus réactionnel vers des produits plus sélectifs.

Par ailleurs, quatre facteurs principaux notamment les matériaux d'électrode, la concentration du substrat, la température de fonctionnement et la tension appliquée ont été étudiés en mode discontinu pour retenir les conditions optimales de production maximale de butanol et de 1,3-propanediol par *C. pasteurianum* dans un système ESM. Le milieu standard sera utilisé en tant que milieu de fermentation en batch. Une idée potentiellement intéressante pour la synthèse d'alcools et de solvants consiste à utiliser un bioréacteur de type double. Un plan central composite a été utilisé pour optimiser les conditions de fonctionnement et pour maximiser la production biochimique dans la gamme expérimentale. Par le fait que *C. pasteurianum* soit une bactérie électroactivée, l'ajout de navette d'électrons pour atteindre des performances plus élevées n'est pas nécessaire. En utilisant les médias de synthèse comme étape initiale de la recherche avec le glucose et le glycérol, puis, en utilisant des déchets réels tels que de glycérol brut, nous avons examiné la production de solvants. La fermentation sans application d'électricité (fermentation conventionnelle) a été également réalisée en guise de comparaison entre le procédé d'ESM et la méthode de fermentation conventionnelle.

En outre, l'électrolyse catalytique a été réalisée pour la formation de produits à valeur ajoutée à partir de glycérol brut. Une nouvelle approche de la conversion du glycérol brut en produits chimiques à valeur ajoutée (le propanediol, l'acrylonitrile, les biocarburants et le gaz de synthèse) suscite un intérêt croissant. Divers procédés peuvent être appliqués pour convertir le glycérol brut en produits chimiques tels que la pyrolyse, la gazéification, l'oxydation sélective, les processus microbiens, l'estérification, l'acétylation, l'hydrolyse et l'hydrogénolyse. Toutefois, ces procédés présentent des inconvénients majeurs : i) faibles rendements ; ii) réactions non sélectives ; iii), coût élevé et ; iv) temps de réaction relativement long. La conversion électrochimique du glycérol brut peut être considérée comme une technologie nouvelle et simple pour générer par voie électrolytique un produit à valeur ajoutée. La conversion électrochimique du glycérol est l'une des méthodes permettant de produire des composés à

haute valeur ajoutée qui n’a pas été suffisamment étudiés dans la littérature. Dans cette étude, nous avons examiné les produits à valeur ajoutée soit le dihydroxyacétone, le hydroxyacétone et le glycidol par conversion électrocatalytique du glycérol pur et brut dans des cellules d'électrolyse. Ceci a été fait en sélectionnant les électrodes les plus appropriées à cette production et en optimisant les différents paramètres de fonctionnement notamment la durée d’opération, la densité du courant et le pH.

Mots-clés : Produit à valeur ajoutée, déchet de nutriments, synthèse électro-microbienne, conversion électrochimique, facteurs effectifs et optimisation.

SUMMARY

Using renewable resources to produce chemicals and fuels is increasing. Chemical and fuel synthesis from the waste in microbial electrolysis cells (MECs) can be emerged as a solution of environmental issues and energy problems, though it has not been commercialized yet. Organic materials from wastes are renewable and serve as alternative sources for the production of biofuels and biochemicals. Nutrient waste, such as crude glycerol could be known as a source of energy which can play a major role in the future of world energy. Using electricity-assisted system in microorganism metabolism (biocatalysts) showed improvement in solvent production compared to conventional fermentation methods. Electrotrophs, the kind of microbial strains which can accept an electron from the cathode to reduce organic carbon source to valuable biochemicals is of interest nowadays. Glucose, glycerol, and other organic compounds could be converted into high value-added bio-solvents, such as butanol, and propanediol. Microbial electrosynthesis (MES) performance is affected by important parameters, such as microbial physiology, materials of electrodes, type of electrolytes and substrates, and applied voltage.

An interaction mechanism between electroactive microorganisms and electrodes via extracellular electron transfer in the presence of excess electrons in the electro-bioreactor is the major aspect of this technology. Since, there are various drawbacks of microbial fermentation of alcohols, solvents, or biofuels, such as low product concentration and productivity, and the high cost of recovery, the electron-assisted process would be an alternative to enhanced yield. Using inexpensive organic materials, and improving fermentation methods by MES, can be used to overcome the current issues. Techno-economic evaluation is also required for MES as it has been carried out for conventional fermentation of biohydrogen and biochemicals. Economic electrode materials with the capability of efficient electron transfer could be carbon-based electrodes which are beneficial due to their good stability and low cost. The special requirements of MES reactor system are an autoclavable reactor, electrodes, and separators (membranes), which should not be neglected in bioreactor design in terms of efficiency, and economic aspects. To sum up, by MES some constraints can be removed in comparison to traditional fermentation and goes towards more selective products.

In this study, the literature review, problems related to alcoholics and solvents production from nutrient waste, objectives, hypothesis and originality, results, discussions, and conclusion are

viewed. Accordingly, four main factors, such as electrode materials, substrate concentration, operating temperature, and poised applied voltage will be investigated in batch mode to achieve optimum condition for producing maximum butanol and 1,3-propanediol by *C. pasteurianum* in MEC. Standard medium will be used as fermentation media in batch operation mode. One potentially interesting idea for alcoholic and solvents synthesis is using an optimal dual-type bioreactor. Numerical optimization using a central composite design (CCD) method will be used to optimize the operating conditions and maximize the biochemicals production within the experimental range. *C. pasteurianum* is an electroactive bacterium and does not need adding electron shuttle for achieving higher performance. We will examine the production of solvents and alcoholics (in microbial electrolysis cell) using synthesis media as the initial step of research (with pure glucose and glycerol) and then by utilizing real wastes such as crude glycerol. Fermentation without applying electricity as the control system is also performed to compare microbial electrosynthesis (MES) and the conventional method.

Moreover, chemical electrolysis (electrochemical conversion of crude glycerol) has been performed to compare the electrosynthesis and bioelectrosynthesis for value-added production from nutrient waste such as crude glycerol. A novel approach for crude glycerol conversion to value-added chemicals, such as propanediol, acrylonitrile, biofuels, or syngas is of growing attention. Various processes can be applied to convert crude glycerol into more valuable chemicals such as pyrolysis, gasification, selective oxidation, microbial processes, esterification and acetylation, and hydrolysis and hydrogenolysis. Such processes have the most important disadvantages: low yield and selectivity, and high cost. Electrochemical conversion of crude glycerol can be emerged as a novel and simple technology to produce value-added product. Electrochemical conversion of glycerol is one of the methods to produce high value-added products which has not been sufficiently studied in previous reports. In this study, we examined the value-added products such as hydroxyacetone, dihydroxyacetone, and glycidol along with organic acids by electrocatalytic conversion of pure and crude glycerol in electrolysis cell by selecting the most suitable electrodes and optimizing the different operating parameters such as operating time, current density, and pH.

Keywords: Value-added product, nutrient waste, electro-microbial synthesis, electrochemical conversion, effective factors, and optimization.

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A	acid HCl concentration
a	exponent of inhibitory product
ABE	acetone-Butanol-Ethanol
Ag/AgCl	silver chloride electrode
ANOVA	analysis of variance
ATP	adenosine triphosphate, is the energy currency of life. Which is found in every cell.
B	butanol concentration (g/L)
BDD	boron doped diamond
b_0	average value of the responses of the assays
b_i	principal effect of each factor *i* on the response
b_{ij}	interaction effect between factor i and factor j on the response
BEC	bio-electrolysis cell
BES	bio-electrosynthesis
Bu20	20% biobutanol and 80% diesel
C	current intensity
CG	crude glycerol
CCD	central composite design
CI	compression ignition
CO	carbon monoxide
DFT	density functional theory
DHA	dihydroxyacetone
DNS	dinitrosalicylic acid
EF	electro-fermentation

Emf	electromotive force
E-BCs	electrobiocommodities
EEB	electron equivalent balance
EET	extracellular electron transfer
EMS	electro-microbial synthesis
G	glycerol concentration (g/L)
GC-MS	gas chromatography mass spectrometry
HPLC	high pressure liquid chromatography
HVAP	high value-added product
K_S	Monod or substrate saturation constant (g/L)
LC-MS	liquid chromatography mass spectrometry
MES	microbial-electrosynthesis
MES	microbial electrosynthesis
MEC	Microbial electrolysis cell
MFC	microbial fuel cell
MECS	microbial electrochemical synthesis
MP2	modified P2 medium
NADH	nicotinamide adenine dinucleotide (reduced form), a coenzyme found in all living cells
NAD^+	nicotinamide adenine dinucleotide (oxidized form)
NR	neutral red
NO_x	nitrogen oxides
OER	oxygen evolution reaction
OA	organic acids
OF	objective function
P	concentration of inhibitory product (g/L)

P^*	critical concentration of inhibitory product above which cells do not grow (g/L)
1,3 PD	1,3 propanediol
Pt	platinum
Pt/Ti	platinized titanium
RSM	response surface methodology
SHE	standard hydrogen electrode
SS	stainless steel
S	solvents (acetol, DHA, glycidol)
S	initial substrate (glucose) concentration (g/L)
S^*	critical glucose concentration above which cells do not grow (g/L)
SMM	standard minimal medium
T	temperature (^oC)
t	time (h)
Ti/IrO_2,	titanium iridium oxide
TN	total nitrogen
TOC	total organic carbon
TSS	total suspended solids
V	voltage (V)
WW	waste water
X	biomass (g)
x_i	coded variable
Y	experimental response

Subscripts

$_{Exp}$	experimental
$_{Cal}$	calculated
m, n	power constants

Greek

μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})
α_0	average value of the responses of the assays
α_i	principal effect of each factor *i* on the response
α_{ij}	interaction effect between factor *i* and factor *j* on the response
β	the number of NADH requirement per mole of metabolism
ΔP	formation of final products in mole or mmole

Première partie

CHAPITRE 1

SYNTHÈSE

1 SYNTHÈSE

1.1 Introduction

En raison de l'épuisement des ressources fossiles, les molécules organiques de faibles masses moléculaires sont devenues des sujets d'intérêt. L'utilisation potentielle de ces molécules, principalement des alcools, est particulièrement intéressante en raison de leur usage dans les piles combustibles d'où l'intérêt de la valorisation chimique/industrielle. Le glycérol est un composé important lié à la biomasse, disponible à la fois en tant que composé polyol et en tant que sous-produit abondant du biodiesel. Plusieurs travaux se sont intéressés à l'investigation des nouvelles applications du glycérol, de par ces dérivés fonctionnels, et ont conclu la possibilité de son utilisation comme matière première à faible coût. Le glycérol peut être utilisé comme additif dans la fabrication du béton et utilisé comme précurseur pour la génération de produits chimiques à valeur ajoutée. Le glycérol peut être également converti par voie catalytique en produits commercialement intéressants tels que la dihydroxyacétone (DHA), l'acétol et les acides organiques. Les réactions du glycérol dans les milieux aqueux sont principalement des réactions redox. Les méthodes électrochimiques sont particulièrement utiles dans l'examen des processus mécanistiques.

En pratique, tous les oxygénats obtenus par oxydation sélective de la glycérine sont commercialement pertinents. Toutefois, la fonctionnalisation extensive d'une molécule de triol-glycérol avec des groupes hydroxyles, de réactivité similaire, rend sa conversion sélective particulièrement difficile. Le glycérol est facilement converti en formaldéhyde, en acide formique et en CO_2 sauf que, dans certains cas, les bilans de masse sont aussi faibles que 20%. En effet l'augmentation de la sélectivité et des taux de conversion sont difficilement accomplis en raison de l'oxydation rapide.

Le glycérol peut être particulièrement intéressant car son oxydation sélective conduit à des produits de forte valeur ajoutée comptant trois groupes hydroxyles. De plus, puisque sa production dépasse de loin sa consommation actuelle, une utilisation alternative est souhaitable, et son utilisation dans les piles combustibles à oxydation directe apparaît comme une option intéressante. En dépit de son assez faible énergie spécifique (4,4 contre 6,1 kWh/kg

pour le méthanol), le glycérol est intéressant par rapport aux autres carburants car il est non toxique, peu volatil, et son transport et son stockage ne sont pas exigeants. Malgré ces avantages, l'oxydation électrocatalytique du glycérol a été moins étudiée que celle d'autres molécules organiques. Néanmoins, au cours de la dernière décennie, quelques études ont été consacrées à l'oxydation du glycérol dans les domaines de la chimie organique, à la catalyse hétérogène avec l'oxygène comme oxydant et à l'électrocatalyse où les électrocatalyseurs ont montré la meilleure activité en milieu alcalin/acide. Différents électrocatalyseurs incluant le palladium, le nickel, l'argent, l'or et le platine se sont montrés électrocatalytiquement actifs pour l'oxydation d'alcool. Des études voltamétriques de l'oxydation du glycérol ont été rapportées pour des milieux à la fois alcalins et acides. De ceci, l'oxydation électrochimique du glycérol sur les électrodes de platine a été un sujet d'intérêt croissant [1-3].

D'autre part, les microorganismes hétérotrophes utilisent un composé organique comme source de carbone par électrofermentation (en utilisant l'électron de la cathode comme équivalent co-réducteur). Durant ces dernières années, on a rarement exploré l'électrosynthèse microbienne de différents produits tels que l'hydrogène, les hydrocarbures, les acides organiques et les alcools. Le type de microorganismes, la concentration des substrats, le mécanisme de transfert des électrons, les paramètres opératoires tels que la température et la tension appliquée, le matériau des électrodes et les configurations du système sont des facteurs clés à cet égard.

1.2 Problématiques

Récemment, la recherche s'est concentrée sur la production de biocarburants et de solvants en utilisant des procédés plus efficaces que les procédés conventionnels telle que la synthèse électromicrobienne (ESM). La fermentation conventionnelle du biobutanol peine d'une faible productivité qui peut être améliorée par la nouvelle technique de synthèse électromicrobienne ou la bioélectrosynthèse (BES). Dans un tel processus, les bactéries catalysent des réactions de réduction des molécules organiques, en utilisant un atome d'hydrogène et un électron de la cathode, pour produire un produit à haute valeur ajoutée. Selon la revue de la littérature précédente et les propos d'autres études, les principaux problèmes soulevés par l'ESM sont mis en avant dans les paragraphes suivants.

La conversion électrochimique du glycérol est l'une des méthodes permettant de produire des produits à haute valeur ajoutée mais qui n'a pas été suffisamment étudiée dans la littérature. La réaction d'hydrogénation catalytique nécessite des températures relativement élevées (250-400 °C) et des pressions d'hydrogène contrôlées, alors que les réductions électrochimiques peuvent se produire à température et pression ambiantes en milieu aqueux où les électrons sont mis à contribution pour des réactions d'oxydo-réduction. D'autre part, le processus d'électrolyse a lieu à pression et à température ambiantes et consomme moins d'énergie par rapport aux approches chimiques. De plus, l'électricité utilisée dans ces réactions peut être fournie grâce à l'énergie solaire (énergie renouvelable).

1.2.1 Microorganismes électroactifs

Le premier problème dans la technologie d'ESM est de trouver les bactéries électroactives. *Clostridium beijerinckii, clostridium acetobutylicum, clostridium saccharobutylicum et clostridium pasteurianum* sont les micro-organismes les plus appropriés pour produire des alcools et des solvants. D'après les données fournies par la littérature, *C. pasteurianum* est la bactérie la plus électroactive pour la production des alcools et des solvants. Cette bactérie peut produire à la fois des alcools (butanol) et des solvants (1,3-propanediol) en utilisant du glucose et du glycérol comme substrats. Des études récentes indiquent que *C. pasteurianum* devrait être utilisée dans les conditions suivantes : pH initial se situant entre 6,8 et 7,0, et une température comprise entre 30 et 37°C [4-6].

1.2.2 Électrodes les plus appropriées

Plusieurs facteurs peuvent affecter les performances de l'ESM tels que la surface spécifique, la biocompatibilité, l'échange d'électrons entre l'électrode et les microorganismes, et la conductivité. Particulièrement, la sélection des matériaux d'électrode dépend des bactéries et des réactions qui ont lieu dans la cellule [7]. Comme le temps de fermentation dépend de la consommation de source de carbone [8] et de la stabilité du biofilm dans l'ESM, le matériau de l'électrode a un effet significatif sur la performance de l'ESM.

1.2.3 Conditions optimales pour une productivité maximale par ESM

Les différents substrats de carbone ont plusieurs voies à suivre pour former le produit final. De multiples paramètres affectent également le rendement des produits finaux. Afin d'atteindre une accumulation maximale, il est souhaitable d'avoir la connaissance de la corrélation entre les paramètres opératoires du réacteur. En effet, à chaque substrat est associé une efficacité et un rendement du facteur d'assimilation. Cette connaissance peut aider à développer un processus plus efficace avec une productivité plus élevée. Puisqu'il existe divers inconvénients de la fermentation microbienne des alcools, des solvants ou des biocarburants, tels que les faibles concentrations et productivité accompagnées de coûts de récupération élevés, un procédé assisté par électrons serait une alternative à rendement élevé. Certes, pour atteindre le coût le plus bas avec une productivité visé et le rendement les plus élevés, le processus doit être optimisé. Cependant, la détermination des conditions optimales (meilleur rendement et productivité maximale), est un défi prépondérant dans le cadre de travail. Plusieurs facteurs qui affectent l'opération d'ESM seront mentionnés dans la section méthodologie.

1.2.4 Conditions de fonctionnement optimales pour la technique de conversion électrochimique

De nos jours, la capacité mondiale de production de biodiesel est en forte croissance. De manière stoechiométrique, le glycérol est produit à 10% en poids de la production totale du biodiesel. L'excédent de glycérol enregistré au cours des dernières décennies en raison de l'augmentation mondiale de la production de biodiesel a créé un nouveau défi en termes de purification du glycérol brut. Le sous-produit brut de glycérol issu du processus de transestérification est considéré comme étant un déchet. D'une part, il peut être stocké dans des fûts et, par la suite, éliminé dans des décharges. D'autre part, étant donné que la vente du glycérol brut n'est pas onéreuse, il est très avantageux d'envisager un nouveau processus pour générer des revenus. En conséquence, certaines études se sont concentrées sur la valorisation ou la production de produits à valeur ajoutée à partir du glycérol brut. Ces travaux portaient sur la conversion du glycérol brut en produits chimiques plus précieux, telles que des molécules riches en fonctionnalités avec trois groupes hydroxyles (-OH) présentant des intérêts industriels.

Par conséquent, des efforts sont déployés pour canaliser la conversion massive du glycérol en des produits chimiques à haute valeur ajoutée. Parmi ces produits chimiques à haute valeur ajoutée, se trouvent l'acétol, la dihydroxyacétone et le glycidol qui ont de nombreuses applications dans l'industrie cosmétique. Il est également possible de citer les intermédiaires organiques pour la production de polyols et d'acroléine, les intermédiaires dans la production de produits pharmaceutiques, les additifs pour les fluides hydrauliques synthétiques et les diluants réactifs dans certains systèmes époxy de résine, etc.

Toutefois, la plupart de ces études ont porté sur le comportement électrocatalytique des électrodes comme le platine, mais ont rarement examiné la conversion électrochimique du glycérol brut en produits à haute valeur ajoutée. Cette conversion électrochimique accompagnée de l'optimisation des conditions de fonctionnement tels que le pH, la concentration d'alimentation et la densité du courant (facteurs les plus importants dans la conception des réacteurs électrochimiques) [9]. Étant donné que les voies d'électrolyse du glycérol ne sont pas étudiées dans leur ensemble et que de nombreux produits peuvent être formés à différentes conditions, l'identification de la voie de réaction et l'optimisation du processus sont nécessaires.

1.3 OBJECTIFS, HYPOTHÈSES, ET ORIGINALITÉ

La production de biodiesel conduit à la production de glycérol brut (10% p/p). Il est issu de la transestérification des acides gras des lipides. Avec la croissance de l'industrie du biodiesel ces dernières années, la disponibilité de glycérol brut a augmenté. Puisque le glycérol brut est considéré comme un déchet, il est nécessaire de développer de nouvelles techniques pour sa réutilisation [10, 11].

La nature fortement réduite du glycérol, différente des sucres tels que le glucose/xylose, conduit à la production de deux équivalents réducteurs par rapport aux sucres. *Clostridium pasteurianum*, en tant que bactérie anaérobie Gram positive, utilise le glycérol comme seule source de carbone et peut produire du butanol, du 1,3-propanediol (1,3-PDO) et de l'éthanol [6].

Les impuretés du glycérol brut sont à l'origine de son prix relativement bas par rapport à celui du glycérol pur. La concentration des impuretés varie en fonction des usines de production de

biodiesel, en raison des différences de charge, du type de catalyseur utilisé dans le processus de transestérification, de l'efficacité et de la récupération du méthanol et des catalyseurs. Ainsi, il est essentiel de comprendre la composition chimique du glycérol brut avant d'effectuer le prétraitement et la fermentation. Généralement, les impuretés sont le méthanol, les acides gras libres (AGL), les sels, l'humidité, la cendre, le savon et les esters méthyliques [12].

Le marché actuel (notammment le marché asiatique) pour le glycérol raffiné est de 600-650 USD/tonne, alors que le glycérol brut coûte 225-235 USD/tonne. En 2015, la production mondiale de biodiesel a augmenté de 13% soit 30 milliards de litres supplémentaires [6].

1.3.1 Hypothèses

Selon les questions soulevées dans la partie 1.2, le présent travail de recherche s'articulera autour des hypothèses suivantes.

1.3.1.1 Hypothèse 1

L'équilibre des intermédiaires redox tel que NADH est une préoccupation cruciale dans l'ingénierie métabolique. À cet effet, l'approche bioélectrochimique est une technique qui permet d'améliorer les équilibres redox dans les systèmes de production de bio-solvants et de biocarburants. La réduction du NAD^+ intracellulaire en NADH peut être effectuée dans un tel système pour équilibrer le redox des cellules. Cette méthode bioélectrochimique peut être utilisée pour améliorer les rendements et/ou la productivité des produits finaux dans le système de fermentation. L'addition des électrons à travers la cathode dans un microorganisme entraînerait une réaction de réduction améliorée en augmentant un cofacteur réduit tel que le NADH comme suit :

Équation 1-1 $$NAD^+ + H^+ + 2e^- \rightarrow NADH$$

Par exemple, *C. pasteurianum* pourrait accepter l'électron de la cathode et par conséquent améliorer la production de produits nets consommant du NADH tels que le butanol (par fermentation du glucose) et le 1,3-propanediol (1,3-PD) par fermentation du glycérol. En effet,

selon la littérature, la consommation totale de NADH pour la production de butanol a montré que la voie métabolique se déplaçait vers la voie de production du butanol par un supplément d'électrons dans ESM, et les résultats indiquaient que la consommation totale de NADH était supérieure à la fermentation témoin. Les bilans d'électrons et de NADH ont révélé que l'électrofermentation pour la production de biocarburants et de produits chimiques peut ouvrir la grande porte vers l'avenir [13, 14].

L'ESM repose sur le transfert des électrons extracellulaires (EEC) [15]. L'EEC est le processus par lequel les bactéries transfèrent des électrons dans et en dehors de la cellule à partir ou à destination d'un donneur/accepteur d'électrons. Au niveau du compartiment anodique, la première hypothèse (idée courante) est le transport d'électrons de la matière organique comme donneur d'électrons vers les minéraux et les électrodes. Le transfert direct des électrons de la souche à l'anode (suite à l'oxydation du substrat) est évident, mais le mécanisme inverse de transport des électrons vers les microorganismes dans la chambre cathodique n'a pas encore été entièrement maitrisé [7]. L'EEC dans la chambre cathodique directe (biocatalyse directe) qui sous-entend un contact direct entre les microorganismes et la cathode, le transfert d'électrons à travers les nanofils et le transport d'électrons par l'hydrogène sont connus pour le mécanisme de transfert des électrons de la cathode aux microorganismes. L'autre option de l'EEC cathodique se fait par navettes électroniques soit par des médiateurs endogènes transportés et des médiateurs artificiels transportés. Finalement, le transfert indirect des électrons se fait via des blocs de construction intermédiaires (sous-produit de navette) tels que le formiate ou via des substances polymères extracellulaires. Le cytochrome C est une protéine localisée dans le compartiment situé entre les membranes interne et externe où il fonctionne pour transférer des électrons de la chaîne respiratoire. Une chaîne de transport d'électrons est une série d'enzymes et de coenzymes qui réalise globalement deux actions simultanément : elle transfère des électrons depuis des donneurs d'électrons vers des accepteurs d'électrons au cours de réactions d'oxydoréduction successives, et elle assure le pompage de protons ou d'autres cations à travers une membrane biologique [14, 16].

1.3.1.2 Hypothèse 2

Les principales conditions d'opération telles que le temps de fonctionnement, la température, la concentration du substrat, la tension appliquée et le matériau de l'électrode déterminent l'efficacité du ESM. D'une part, le faible taux de charge organique diminue les quantités de production. D'autre part, une concentration élevée de substrat peut épuiser la capacité de production en raison de l'effet d'inhibition. À l'instar de ceci, une faible tension appliquée entraîne une production faible, ce qui n'est pas avantageux pour obtenir un rendement élevé. Cependant, une haute tension peut conduire à l'absence de sous-produit ou endommager les microorganismes. Une longue durée de fonctionnement peut conduire à un gaspillage d'énergie en raison de l'épuisement du substrat ou de l'arrêt de la production en raison de l'inhibition. Aussi, les électrodes à base de carbone sont largement utilisées de par leur conductivité qui est relativement faible par rapport aux électrodes métalliques. Cependant l'utilisation de collecteurs métalliques peut compenser cet inconvénient. Les électrodes métalliques comme le titane et l'acier inoxydable ne semblent pas convenir en raison de leur surface active plus faibles et de leurs propriétés superficielles, ce qui réduit le développement du biofilm [17].

1.3.1.3 Hypothèse 3

Différents substrats de carbone ont de multiples voies réactionnelles pour finalement produire des produits finaux. L'ESM peut être appliquée pour différents déchets y compris les sucres et le glycérol. La performance globale en termes d'efficacité, de productivité de viabilité économique sont les principaux facteurs déterminant le choix du processus. Le glucose et le glycérol peuvent être utilisés comme source de carbone pour la production de biosolvants et de biocarburants alcooliques. Cependant, l'utilisation de substrats purs n'est pas rentable du point de vue économique. Les autres alternatives (tel que le glycerol brut) peuvent être utilisées pour la fermentation. En outre, les déchets doivent être prétraités avant la fermentation comme décrit dans la section méthodologie.

De nombreux rapports ont mis en relief l'importance du rapport C:N. Il est donc important de le maintenir au niveau requis dans le réacteur. Le rapport C:N optimal peut être déterminé par diverses expériences d'optimisation en modifiant la concentration d'alimentation. Certes, un

rapport C:N approprié pour la culture pure est nécessaire pour optimiser la production chimique à partir du substrat organique [18].

1.3.1.4 Hypothèse 4

Stoechiométriquement, le glycérol est produit à 10% en poids et ce, en considérant la production totale du biodiesel. Cependant, la conversion du glycérol pur en composés à valeur ajoutée par voie électrochimique sans utilisation de microorganismes a été rapportée [19]. Le présent travail tente de transformer le glycérol brut en composés à valeur ajoutée par la technique électrochimique. Le glycérol enrichi obtenu par prétraitement peut être utilisé comme substrat brut pour la synthèse électrochimique d'autres composés. L'enrichissement du glycérol brut peut être utilisé en vue d'optimiser le rendement et la production. Particulièrement dans des conditions très acides (pH d'environ 1), les pics de réduction et d'oxydation du glycérol brut enrichi, dont l'amplitude est importante, ont été observés. La conversion électrochimique du glycérol en produits chimiques tels que l'acétol, le dihydroxyacétone et le glycidol, peut être développée dans un système électrochimique simple. Chaque produit visé présente une densité de courant spécifique et une durée d'électrolyse optimale correspondante.

1.3.2 Objectif principal

L'objectif principal de cette recherche porte sur la production de butanol et de propanediol par électrosynthèse microbienne, suivie d'une étude comparative avec la méthode de fermentation conventionnelle. À cela, s'ajoute l'étude portant sur la production de l'acétol et du dihydroxyacétone par conversion électrocatalytique à partir du glucose et du glycérol respectivement utilisés comme source de carbone.

Les objectifs spécifiques suivants seront considérés pour surmonter les problèmes mentionnés.

1.3.3 Objectifs spécifiques

En utilisant des substrats, la technologie de fermentation conventionnelle au niveau commercial présente plusieurs contraintes notamment le milieu de culture spécifique à chaque microorganisme (dans la plupart des cas), le déséquilibre redox limitant la sélectivité du produit et le contrôle du pH limitant souvent l'application et la durabilité. L'ESM constitue un moyen

approprié pour l'utilisation de la biomasse des déchets agro-industriels vers des bioraffineries. En utilisant l'ESM, le processus de fermentation peut être contrôlé et optimisé pour obtenir des produits de pureté plus élevée. L'ESM permettrait de contrôler plus facilement les paramètres opératoires (pH, régulation redox, agents antimousses, etc.) lors de la fermentation de divers types de déchets de type organique [20].

1.3.3.1 Objectif 1 : ESM et étude des mécanismes

L'un des premiers objectifs spécifiques de ces travaux a été d'identifier les bactéries les plus appropriées pour l'électrosynthèse microbienne, suivi de l'électro-fermentation dans un bioréacteur de type H (chambre double) en utilisant des milieux synthétiques (contenant respectivement du glucose et glycérol). Cette première partie de l'étude visait également à réaliser une étude comparative entre l'électrosynthèse microbienne et la fermentation conventionnelle sans électricité, ainsi que la compréhension du mécanisme de métabolisme de fermentation par NADH. La consommation de NADH peut être calculée par l'équation suivante [13] :

Équation 1-2 $$NADH^{cons} = \beta \Delta P$$

Où β est le nombre de NADH requis par mole de métabolisme, ΔP est la formation de produits finaux en mole ou en mmole.

1.3.3.2 Objectif 2 : Électrode la plus appropriée

Le second objectif consiste à tester les électrodes (électrode de carbone versus électrodes métalliques) les plus appropriées pour l'ESM. Plusieurs facteurs peuvent affecter les performances d'ESM tels que la surface spécifique, la biocompatibilité, l'échange d'électrons entre l'électrode et les microorganismes et la conductivité électrique de l'électrode. La sélection du matériau des électrodes dépend des bactéries et des réactions qui ont lieu dans la cellule [7]. Comme le temps de fermentation dépend de la consommation de sources de carbone [8] et de la stabilité du biofilm dans l'ESM, le matériau de l'électrode a un effet significatif sur la performance de l'ESM.

1.3.3.3 Objectif 3 : Optimisation et corrélation

Le troisième objectif est d'optimiser les conditions de fonctionnement telles que la température, la concentration substrat/charge et la tension appliquée. La corrélation entre la concentration des produits finaux et les paramètres opératoires sera également réalisée.

1.3.3.4 Objectif 4 : ESM de déchets réels

Le quatrième objectif consiste à valider les performances de l'ESM sur des déchets réels, tel que le glycérol brut pour produire des produits à haute valeur ajoutée. L'utilisation des substrats purs n'est pas économiquement rentable.

1.3.3.5 Objectif 5 : Production à valeur ajoutée par électrosynthèse et étude des mécanismes

Des expériences seront effectuées dans une cellule d'électrolyse pour produire des produits à haute valeur ajoutée à partir de déchets contenant des matières organiques comme le glycérol brut et pure, respectivement. Cette étude peut explorer la capacité de l'électrolyse chimique des déchets. Les expériences préliminaires ont pour but d'explorer la distribution du produit, les conditions de fonctionnement, les matériaux utilisés pour les électrodes, le pH de la solution, le mécanisme et la cinétique des réactions.

1.3.3.6 Objectif 6 : Optimisation du processus électrocatalytique

Étant donné que ce processus est très sensible au courant appliqué, au matériau de l'électrode utilisé comme catalyseur et au pH de la solution, il est nécessaire d'optimiser le processus pour obtenir le taux de production maximale des produits chimiques souhaités. À partir de l'électrolyse du glycérol, les produits acides et non acides peuvent être obtenus. Dans cette étude, notre objectif est de produire un maximum de produits non acides, car ils ont une valeur ajoutée supérieure à celle des produits acides.

1.3.4 Originalité

Selon la revue de la littérature, la plupart des études dans le domaine d'ESM sont liées à la production de bio-hydrogène et à la pile à combustible microbienne (production d'électricité) et quelques études sur la production d'alcools et de biocarburants. De plus, il n'y a aucun article publié qui traite de la production de solvants dans des conditions optimisées et à partir de déchets réels. Cette recherche se concentre sur la variation des paramètres au cours de la production chimique dans diverses conditions de traitement ce qui lui confère un caractère original et unique. L'étude aboutira à corréler les effets de divers paramètres du processus à la concentration des produits finaux. En outre, l'originalité du projet proposé est la production optimale de composés à valeur ajoutée à partir de déchets synthétiques et réels, par la conversion électrochimique et l'électrolyse microbienne.

1.4 Méthodologie

À la lumière des objectifs prédéfinis, la méthodologie suivante sera réalisée.

1.4.1 Bioélectrosynthèse (BES) en utilisant des milieux synthétiques (Microorganismes et milieux de culture)

Choi et al. [13] ont montré que parmi *C. pasteurianum, C. acetobutylicum et C. tyrobutyricum, seul C. pasteurianum* présentaient le pic de réduction le plus approprié pour la BES. En conséquence, *C. pasteurianum* ATCC 6013TM a été fourni par la compagnie CEDARLANE (American Type Culture Collection). Le microorganisme lyophilisé a été relancé et cultivé (après un choc thermique à 80 °C) dans des milieux peptone-extrait de levure-glucose (PYG) en condition anaérobie pendant 36 h à 37 ± 0,01 °C et 150 tpm dans des flacons de sérum de 125 mL (volume utile 50 mL). Le milieu (g/L) était composé de glucose (10), extrait de levure (10), peptone (5), tryptone (5), cystéine-HCl (0,5), K_2HPO_4 (2,04), KH_2PO_4 (0,04), $FeSO_4$-$7H_2O$ (0,0011), $CaCl_2$ (0,008), $MgSO_4$-$7H_2O$ (0,0192), NaCl (0,08) et $NaHCO_3$ (0,4). Tous les produits chimiques ont été achetés auprès de Fischer Scientific et étaient de qualité analytique. Avant la fermentation, le SMM pour les cultures discontinues (fermentation) a été blanchi dans dans 1 litre d'eau distillée comprenant (g) K_2HPO_4 (1,74), NH_4Cl (0,66-2), $MgSO_4$-$7H_2O$ (0,251), KCl (0,596), $NaHCO_3$ (6), acide p-aminobenzoïque (0,004), biotine (0,00024), extrait de levure

(0,5), résazurine (0,001), cysteine-HCl (0,5), et le glucose entre 20 et 200 g. Le pH a été ajusté à 6,7 avec du KOH. Le milieu MP2 a été également testé comme milieu de fermentation contenant (gramme par litre d'eau distillée): glucose (20), K_2HPO_4 (0,5), KH_2PO_4 (0,5), $MnSO_4$-H_2O (0,01), $MgSO_4$ -$7H_2O$ (0,02), $FeSO_4$-$7H_2O$ (0,01), extrait de levure (1), (NH_4) 2-SO_4 (2), NaCl (0,01), biotine (0,01 mg), thiamine (1 mg) et acide p-aminobenzoïque (1 mg). Les milieux contenaient également 100 mM d'acide 2- (N-morpholino) éthanesulfonique pour empêcher la suracidification [21].

Avant de commencer la BES, la souche a été cultivée dans des flacons de sérum de 125 mL (volume de travail 50 mL) à pH 6,7. Après avoir injecté du gaz neutre pour éliminer l'oxygène dissous dans le milieu, le flacon de sérum est immédiatement fermé avec un joint à sertir en aluminium contenant du septum de silicone (Fisher Scientific, Canada) au moyen d'une pince à sertir manuelle (E-Z Crimper TM, VWR, Ontario, Canada). Ensuite, il a été incubé (INFORS HT) à 37 °C et à 150 tr / min pendant environ 20 h pour atteindre la phase exponentielle de croissance. Pour effectuer des expériences d'ESM, les milieux ont été stérilisés pendant 20 minutes à 121 °C. Environ 5-10% (v/v) de culture microbienne dans sa phase exponentielle de croissance (pendant environ 20 heures où le taux de croissance a été mesuré par OD 600 (BioTek) a été inoculé dans la chambre cathodique. Dans le milieu, l'oxygène a été purgé avec N_2 gazeux (groupe Linde) pendant 10 min avant l'inoculation.

1.4.2 Configuration du bioréacteur et système d'électro-fermentation

Un réacteur de type H à double chambre (Adams & Chittenden Scientific Glass) comprenant deux chambres de 300 mL a été utilisé. Il était équipé de membranes échangeuses de cations Nafion 117 (Les membranes Nafion sont fabriquées à partir d'un polymère à base de polytétrauoroéthylène persulfoné (PTFE)) qui présente une stabilité chimique et thermique très élevée. Il comprend un groupe d'acide sulfonique qui fournit des sites pour le transport de protons), les deux électrodes (cathode et anode) étaient en feutre de graphite (Electrolytica, Inc Precise Town et Country) avec les caractéristiques suivantes : précurseur matériau (fibre de polyacrylonitrile PAN), superficie de 0,7 m^2/g, teneur en carbone 99,7%, épaisseur de fibre individuelle 10-20 μm. De plus, la cathode en acier inoxydable 304 (Rodrigue Métal Ltée Québec, Canada) a été testée dans une chambre cathodique pour comparer les matériaux à

base de métaux et ceux à base de carbone. Le générateur de la série PWS2000, Tektronix a été utilisée pour la production d'électricité linéaire à tension constante). L'électrode de référence était Ag/AgCl 3M NaCl qui a été immergée dans la chambre cathodique. De même, un multimètre (Fluke-117 True RMS) a été utilisé pour mesurer le potentiel entre la cathode et l'électrode de référence. Le réacteur a été immergé dans un bain d'eau équipé de circulateurs à immersion chauffants (Polystat) pour fonctionner à température et à pH constants. Le pH pouvait être ajusté en ajoutant du KOH au système.

Après autoclavage du réacteur, le compartiment anodique a été rempli avec une solution tampon de potassium 0,1M (pH neutre) et le compartiment cathodique a été rempli de MP2 ou de SMM. Pour préparer le milieu, trois solutions ont été préparées séparément. La première contenait du glucose et un extrait de levure comme sources de carbone et d'azote. Elle a été autoclavée à 121 °C puis refroidie avant sont utilisation. La deuxième était une solution tampon faite de potassium. La troisième contenait des vitamines et des minéraux. La deuxième et la troisième solutions ont été ajoutées à la première solution après leur filtration (filtres stérilisés dont le diamètre est de 0,2 µm). La fermentation est réalisée pendant 72 h à 37,00 ± 0,01 °C de température pour comprendre initialement le mécanisme du système, puis à différentes températures (30-37°C) afin d'optimiser le système. Les milieux sont constamment agités à 150 tr/min par un agitateur magnétique (MS-12BB, JEIO Tech Co., LTD, Corée du Sud). Le pH est ajusté à 6,7 à l'aide KOH au début du processus de fermentation. Des densités de courant élevées peuvent être atteintes dans une solution contenant des concentrations élevées de solution tampon (K_2HPO_4 et KH_2PO_4), ce qui entraîne une perte ohmique plus faible en raison de la conductivité ionique améliorée [22].

Dans le BES, sur la surface anodique, des ions hydrogène (protons) sont produits (conditions abiotiques) et passent à la cathode, dans le compartiment cathodique, via la membrane d'échange. Les microorganismes utilisent des électrons et des ions hydrogène avec le substrat dans la réaction biotique de réduction (où les électrodes étaient connectées à une source électrique). Contrairement à la pile à combustible microbienne, ce système utilise des électrons pour générer plus de produits à valeur ajoutée. Simultanément, la fermentation a été réalisée sans utiliser d'énergie électrique comme système de contrôle. Chaque expérience a été réalisée en duplicata ou en triplicata.

1.4.3 Échantillonnage et analyse

Des échantillons liquides ont été prélevés régulièrement dans la chambre cathodique (ESM) à l'aide d'une seringue stérilisée. Le glucose en tant que sucre réducteur a été mesuré en utilisant la méthode DNS comprenant : la préparation du blanc (1 mL d'eau + 2 mL de réactif DNS) et l'échantillon (1 mL d'échantillon + 2 mL de réactif DNS). Après un bon mélange et une ébullition pendant 5 minutes, les échantillons ont été refroidis pendant 30 minutes. Puis, après ajout de 7 mL d'eau, des mesures de l'absorbance ou de densité optique ont été effectuées à une longueur d'onde de 540 nm (pic d'absorbance enregistré à cette longueur d'onde) à l'aide d'un spectromètre modèle Carry UV 50 (Varian Canada Inc.)

Le butanol, l'éthanol, l'acétone, l'acide lactique, l'acide acétique et l'acide butyrique ont été analysés par chromatographie en phase gazeuse (GC 7890B, Agilent Technologies, USA) équipée du détecteur FID, avec une colonne HP-INNO-Wax (30 m, 0,25 mm ID et 0,25 um df). Avant l'analyse par GC, les échantillons ont été centrifugés (à 8000 g pendant 10 min) pour séparer la biomasse.

Un chromatograph en phase gazeuse (modèle Varian 3800, USA) muni d'un détecteur de conductivité thermique (TCD) équipé d'une colonne PoraPLOT Q® de 3 m (technologie Agilent, USA) a été utilisée pour l'analyse des échantillons gazeux.

La quantification du glycérol a été effectuée en utilisant la méthode de chromatographie liquide (HPLC) (Finnigan Surveyor LC Pump Plus et Autosampler Plus) ayant les paramètres suivants; colonne : Hypersil Gold Amino 150 x 2,1 mm x 3 µm, four : 35 ^{o}C, débit : 0,25 ml/min, volume injecté : 10 µl, température du plateau : 25 °C, pendant 11 minutes, et pression: 2200 psi. L'azote total (TN) du glycérol brut a été mesuré à l'aide de l'instrument Shimadzu VCPH, méthode TN Curve 0-5 mg/L, avec une limite de détection de 0,02 mg/L et le carbone organique total (COT) a été déterminé par la courbe NPOC (carbone organique non purgeable) 0-5 méthode mg/L. La détermination des matières en suspension, selon la méthode analytique (MA 115 - SS 1.2), a été effectuée en filtrant sous-vide une portion d'échantillon (normalement 100 mL) à travers un filtre en microfibre de verre Whatman 934 AH préalablement séché et pesé et dont la taille des pores était de 1,5 µm. Une fois la filtration terminée, le résidu a été séché à 103-105 °C pendant une nuit ou au moins 3 heures. Le poids des solides en suspension a été obtenu en calculant la différence entre les poids des filtres avant et après séchage.

Le glycérol brut traité a été d'abord identifié en utilisant un balayage par chromatographie en phase gazeuse GC-MS-Thermo Scientific, modèle ISQ, colonne : DB-WAX, diamètre 30 mm x 0,2 mm x 0,2 um d'épaisseur. Ceci en appliquant le flux de colonne 1,0 mL/min (gaz vecteur : hélium), mode d'entrée frontale GC: Splitless, température d'entrée frontale GC : 225 °C et durée: 34,8 min.

Des échantillons liquides ont été prélevés régulièrement de la chambre. La concentration des produits en solution, notamment l'acétol, le DHA et le glycidol, a été déterminée par chromatographie en phase liquide selon la méthode LC-MS-MS Thermo TSQ Quantum.

Les cations (métaux) ont été déterminés par ICP-OES axial Vista Pro, puissance: 1,30 kW, débit du nébuliseur : 0,85 L/min, temps de lecture par réplique : 10 s.

1.4.4 Bioélectrosynthèse (BES) utilisant des déchets réels

1.4.4.1 Pré-traitement

Les déchets réels tels que le glycérol brut doivent être traités avant l'électrofermentation en raison des impuretés présentes et des produits chimiques non fermentescibles. Pour pré-traiter le glycérol brut, il est tout d'abord homogénéisé par le biais d'agitation. Ensuite, il est mélangé avec de l'eau déminéralisée (2 litres d'eau déminéralisé pour 1 litre de glycérol brut) pour l'obtention d'une solution aqueuse. La solution obtenue est ensuite filtrée par des filtres cellulosiques (rétention des particules>25 µm-Fischer Scientific), puis diluée 10 fois et filtrée avec un filtre de 0,22 µm pour éliminer principalement les fines particules solides. Les mesures analytiques sont effectuées sur la solution diluée [6].

Dans une autre approche, le glycérol brut (GB) est un déchet liquide hétérogène visqueux qui a un aspect de type gel et une couleur brun foncé. Les GB de l'usine de fabrication de biodiesel contiennent des impuretés, telles que le méthanol et le savon, et peuvent inhiber la croissance microbienne. Étant donné que le méthanol s'évapore à 65 ± 1 ^{o}C, sa stérilisation peut éliminer une quantité importante de méthanol. Le glycérol brut est homogénéisé par agitation puis le glycérol brut est mélangé avec environ le double d'eau dé ionisée pour une solution aqueuse. Ensuite, d'autres nutriments (sels minéraux, source d'azote, etc.) sont ajouté pour ajuster le niveau d'éléments nutritifs au niveau désiré [23, 24].

1.4.4.2 Électro-fermentation des solutions prétraitées

1.4.4.2.1 Glycérol brut

L'électrosynthèse microbienne a été réalisée dans des milieux standards comme mentionné dans les sections précédentes. Le glycérol brut prétraité a été utilisé en lieu et place du glycérol pur. Divers paramètres ont également été étudiés.

1.4.5 Électrosynthèse (conversion électrochimique) du glycérol pur et des déchets réels (glycérol brut)

Le glycérol brut purifié, ayant une pureté supérieure à 96%, peut être utilisé dans les industries alimentaires, pharmaceutiques ou cosmétiques, mais ces marchés restent limités. En conséquence, une nouvelle approche pour la conversion du glycérol brut en produits chimiques à valeur ajoutée tels que le propanediol, l'acrylonitrile, les biocarburants ou le gaz de synthèse, suscite de plus en plus d'intérêt. Divers procédés peuvent être appliqués pour convertir le glycérol brut en produits chimiques à haute valeur ajoutée tels que la pyrolyse, la gazéification, l'oxydation sélective, les procédés microbiens, l'estérification, l'acétylation, l'hydrolyse et l'hydrogénolyse. De tels procédés présentent certains inconvénients à savoir les faibles rendements et sélectivités, le coût élevé et le temps de traitement relativement long. Or, la conversion électrochimique du glycérol brut peut être considérée comme une technologie nouvelle et simple pour produire un produit à valeur ajoutée. Par cette technique, le glycérol brut enrichi peut être converti en composés à valeur ajoutée, tels que l'acétol et le dihydroxyacétone. La solution initiale de glycérol, le pH, le matériau de l'électrode et la densité de courant/tension appliquée sont des paramètres à optimiser pour une meilleure efficacité du procédé. Chaque produit a une tension appliquée optimale spécifique ainsi qu'un temps d'électrolyse [19]. Les essais ont été effectués dans des cellules d'électrolyse construites en laboratoire. Les cellules (capacité de 0,5 L) étaient en plexiglas (17,7 × 2 × 15,2 cm). Les électrodes ont été installées verticalement sur une plaque de plexiglas perforée placée à 2,0 cm du fond de la cellule et la distance entre les électrodes était de 1 cm. Le mélange dans la cellule a été réalisé par un barreau d'agitation recouvert de téflon qui a été installé entre la plaque perforée et le fond de la cellule. Un courant électrique a été appliqué via un générateur de courant continu Xantrex XFR40-70 (Aca Tmetrix, Mississauga, Canada), avec un courant

nominal maximal de 70 A à un potentiel de circuit ouvert de 40 V. Les expériences ont été effectuées en mode batch (cuve agitée). La tension appliquée entre l'électrode de travail et l'électrode de référence (Ag/Agcl) était constante. Des essais ont également été effectués à intensité de courant constante. Les trois types d'électrodes (Pt, Pt-Ti, et Pt au carbone) ont été testés sous différentes intensités de courant (0,1-1 A) et différents pH dans des conditions acides et basiques (ajustement avec HCl et NaOH). Finalement, la conversion, la sélectivité, le rendement et le mécanisme des réactions (oxydation, réduction et déshydratation) ont été discutés.

1.4.6 Analyse statistique et optimisation

Afin d'optimiser les paramètres de fonctionnement, une méthodologie de surface de réponse (RSM) a été considérée. La RSM est une collection de méthodes mathématiques et statistiques pour la modélisation, l'optimisation et l'analyse d'un processus de traitement dans lequel la réponse peut être influencée par plusieurs variables. Au nombre de cette collection de méthodes, se trouve le plan central composite (CCD) souvent utilisé pour l'optimisation du processus de traitement. La température, le pH, la tension appliquée, la quantité d'inoculum, la concentration du substrat (glucose, glycérol, etc.) et le matériau de l'électrode ont été testés pour étudier leurs effets sur la vitesse de production en ESM. En outre, l'analyse de variance (ANOVA) est effectuée pour déterminer la signification de chaque paramètre. Ceci a montré que la concentration de glucose, la température, le pH, la tension appliquée, et le type d'électrode (cathode) sont les paramètres les plus déterminants. Une conception composite centrale (CCD) a été appliquée pour évaluer et déterminer les conditions opératoires optimales pour la production maximale de butanol. Des expériences ont été effectuées en imposant différentes concentrations de substrat, différentes températures, et divers potentiels de tension pour déterminer les conditions optimales de production de butanol à pH constant et avec des électrodes appropriées. Si la concentration du substrat est supérieure à une valeur définie, les concentrations de produit et de microorganismes n'augmenteront pas avec un substrat supplémentaire, ce qui entraînera une perte de ressources et d'énergie [25]. De plus, pour la conversion électrochimique du glycérol, le pH joue un rôle important. Par ailleurs, la tension appliquée et le type d'électrode sont les autres facteurs qui doivent être considérés pour

optimiser le processus. Il est possible de déterminer l'effet (b) de chaque facteur (X) ainsi que l'effet de leurs interactions (X_iX_j) sur la réponse (Y). Ceci est bien illustré dans le modèle polynomial suivant:

Équation 1-3 $$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{11}X_1^2 + b_{22}x_2^2 + b_{33}X_3^2$$

Où Y est la réponse expérimentale; b_0 représente la valeur moyenne des réponses des dosages; X_i la variable codée; b_i représente l'effet principal de chaque facteur i sur la réponse et b_{ij} représente l'effet d'interaction entre le facteur i et le facteur j sur la réponse (Y). Le logiciel Design-Expert Program (Design Expert 7, Stat-Ease Inc., Minneapolis) a été utilisé pour calculer le coefficient du modèle polynomial. Afin d'évaluer la signification de ces modèles, l'analyse de variance (ANOVA) a été réalisée par le logiciel expert. Un coefficient de corrélation supérieur à 0,8 indique que les modèles sont acceptables avec une bonne corrélation entre les valeurs prédites et expérimentales. Ceci est également confirmé en comparant Pr>F avec la valeur α (α = 0,05) où Pr> F devrait être inférieur à α pour que les modèles soient acceptés.

1.4.7 Consommation de NADH et balance équivalente d'électron

La performance du BES est affectée par certains paramètres importants, tels que la physiologie microbienne, le matériau des électrodes, les types d'électrolyte et de substrat, le potentiel redox, la configuration du réacteur, le pH et la température (par oxydation en NAD^+) pour chaque mole de butanol produite à partir d'acétyl-CoA [26].

Au cours de l'électrofermentation, le métabolisme se déplace vers une production de composés, conduisant à la distribution de produits affectés par le flux de carbone et d'électrons. Ainsi, le ratio électrons/flux de carbone peut être un facteur décisif pour l'appoint final des bioproduits. La consommation de NADH a entraîné une augmentation de la production de butanol en raison des changements nets de NADH respectivement de -2 et de 0 par rapport au butanol et au butyrate. La consommation de NADH peut être calculée à l'aide de l'équation suivante : $NADH^{cons} = \beta\Delta P$ où β est le nombre de NADH requis par mole de métabolisme.

Pour reconnaître comment le flux des électrons a changé dans le métabolisme de fermentation du butanol dans les cultures témoin et BES, l'équilibre des électrons doit être atteint avec les produits finaux.

Une mole de butyrate, d'acétate, d'éthanol, d'acétone et de butanol est égale à 20, 8, 12, 16 et 24 e^- respectivement [26]. La distribution électronique du substrat et des produits peut être calculée en multipliant l'équivalent par moles de chaque composé. L'électrode (dans ce cas la cathode) peut agir comme source d'électrons, ce qui conduit à une fermentation déséquilibrée. Le transfert d'électrons à partir de la cathode peut modifier le milieu en modifiant l'équilibre de la réaction d'oxydo-réduction. Ce mécanisme peut être utilisé pour résoudre certaines contraintes de fermentation classiques. Par exemple, des améliorations majeures de la production de produits tels que le 1,3-propanediol ou le butanol ont été observées [27].

Clostridium beijerinckii est une bactérie à Gram positif ayant une faible capacité de transfert d'électrons extracellulaires (EEC). Il n'est pas un microorganisme électroactif comparé à *C. pasteurianum* connu comme une bactérie électroactive [13]. Par conséquent, les transporteurs d'électrons ont été utilisés, tels que le rouge neutre (NR), pour améliorer la production de butanol par *C. beijerinckii*. Par rapport à *C. pasteurianum, C. beijirinkii* a besoin de médiateurs électroniques (désavantages) mais a plus de productivité à une concentration de glucose plus faible (avantages). Ainsi, l'application du potentiel pour ce système a juste conduit à une durée de fonctionnement plus courte et à aucune amélioration du produit final [28]. En outre, Zhange et al [29] ont montré que les acides organiques tels que le butyrate et l'acétate augmentaient au début de la fermentation mais diminuaient ensuite. Cependant, les résultats opposés ont été obtenus dans le présent travail sauf pour l'acide lactique.

1.5 Principaux résultats et discussion générale

Les nutriments issus de la biomasse dans les eaux usées sont des sources renouvelables et alternatives pour la production de combustibles et de produits chimiques. Les déchets ayant des éléments nutritifs tels que le glycérol brut (sous-produit de la production de biodiesel), pourraient être considérés comme une source d'énergie pouvant jouer un rôle majeur dans l'avenir de l'énergie mondiale. Certains produits chimiques (tels que les alcools) peuvent être générés en combinant la fermentation microbienne et l'électrolyse. Le mécanisme d'interaction

entre les microorganismes électroactifs et les électrodes via le transfert d'électrons extracellulaires et le métabolisme intercellulaire en présence d'excès d'électrons dans l'électro-bioréacteur ont été également étudiés. Étant donné les inconvénients de la fermentation microbienne des alcools, des solvants ou des biocarburants (faible productivité du produit et coût relativement élevé de récupération), le procédé de fermentation biologique assisté par électrolyse serait une alternative intéressante pouvant permettre de majorer le taux de production. Aussi, l'utilisation de matières organiques peu coûteuses, l'amélioration des méthodes de fermentation (par exemple l'ESM) et la récupération efficace des produits sont connues. L'utilisation de médiateur d'électrons (barrière d'électrons) est une autre alternative pour améliorer le rendement et la productivité. Les techniques de co-culture (culture de deux types de cellules ou plus dans une culture où un microorganisme agit comme agent réducteur pour l'autre bactérie qui améliore l'efficacité de la production), la modification génétique des bactéries (espèces Clostridia pouvant tolérer une concentration élevée de butanol), la séparation *in situ* des produits sont d'autres options pour améliorer la fermentation qui peut être combinée au ESM afin d'obtenir un rendement plus élevé et de minimiser les coûts de production. Une évaluation technico-économique est également requise pour les ESM. La synthèse de produits chimiques à partir de déchets dans les ESM peut être considérée comme une solution aux problèmes environnementaux et aux problèmes énergétiques, bien qu'elle n'ait pas encore été commercialisée. La littérature a montré que les électrodes économiques ayant une capacité de transfert d'électrons efficace pourraient être des électrodes à base de carbone qui sont bénéfiques en raison de leur bonne stabilité et de leur faible coût. On peut également en conclure que les réacteurs, les électrodes et les séparateurs autoclavables (membranes) ne doivent pas être négligés dans la conception des bioréacteurs en termes d'efficacité et d'aspect économique. Comme l'ESM pour les biocarburants alcooliques en est à ses balbutiements (d'importantes études ont été menées sur la production d'hydrogène), la production de solvants et d'alcools dans les ESM par le biais de milieux synthétiques et de déchets réels est en cours. En résumé, certaines contraintes peuvent être surmontées par rapport à la fermentation traditionnelle.

Dans les processus thermochimiques, le contrôle de la sélectivité et l'augmentation de l'efficacité nécessitent une modification de la température et de la pression, ce qui entraîne une augmentation significative des coûts de production. Sinon, les processus électrochimiques ont

le potentiel d'utiliser de l'électricité provenant de sources renouvelables. En comparaison avec les technologies chimiques, les processus électrochimiques peuvent supprimer le besoin en équipement de chauffage ou de pressurisation du réacteur. De plus, il est possible de modifier les sélectivités en régulant les potentiels appliqués, le pH des électrolytes et en sélectionnant les électrocatalyseurs pour activer les liaisons ciblées.

1.5.1 Électrofermentation du glucose en utilisant *C. pasteurianum*

L'électrosynthèse microbienne des solvants et la production d'acides organiques ont été effectuées en fermentation discontinue pendant 72 h. L'expérience initiale utilisant 20 g/L de glucose a montré que *C. pasteurianum* pouvait agir comme une bactérie appropriée dans l'ESM et améliorer la production de butanol et la réduction des acides organiques. Diverses concentrations de glucose dans une plage de 20 à 140 g/L ont montré que la concentration en glucose supérieure à 120 g/L dans le milieu de fermentation entraînait un effet d'inhibition mesure le taux de croissance spécifique. La cathode (feutre de graphite) a été utilisée comme donneur d'électrons pour la production de butanol. Les résultats ont montré que cette technique permet non seulement d'augmenter considérablement le butanol, mais que l'acide butyrique (produit à valeur ajoutée) peut également être généré. De plus, *C. pasteurianum* est une bactérie active (pas nécessaire d'ajouter une navette d'électrons, des molécules organiques qui peuvent être oxydées et réduites de manière réversible, conférant ainsi la capacité de servir de porteurs d'électrons parmi de multiples réactions redox) pour obtenir de meilleures performances. Une idée potentiellement intéressante pour la synthèse industrielle du butanol consiste à utiliser un bioréacteur de type double chambre. La réaction de synthèse du butanol a été étudiée et optimisée (en utilisant la méthodologie de surface de réponses : plan central composite) pour maximiser le taux de production de butanol. La production globale pendant trois jours de fonctionnement a été considérée comme un critère d'optimisation. De plus, trois variables ont été choisies notamment la quantité de substrat dans le bouillon de fermentation, le contrôle de la température (à 33,51 ^{o}C) et la tension en veille (1,32 V) en utilisant du feutre de graphite comme électrode à pH constant (6,7) et avec une vitesse d'agitation de150 tr/min.

1.5.2 Électrofermentation du glycérol en utilisant *C. pasteurianum*

L'EMS de production de solvants et d'acides organiques a été réalisée en mode de fermentation discontinue. *C. pasteurianum,* en tant que microorganisme hétérotrophe électroactif, peut agir comme une bactérie appropriée dans le système EMS tout en améliorant la production de 1,3-PD. Dans cette étude, l'opération EMS a été étudiée et optimisée pour maximiser le taux de production de 1,3-PD en utilisant du glycérol brut traité. La production globale pendant deux jours de fonctionnement a été considérée comme un critère d'optimisation. Aussi, la concentration de substrat dans le bouillon de fermentation (41,3 g/L de glycérol), la température (à 33,9 ºC) et la tension au repos (1,6 V), en utilisant du feutre de graphite comme électrode à pH initial (6,7) et à vitesse d'agitation (150 tr/min) ont abouti à 7,42 g/L de 1,3-PD.

1.5.3 Conversion électrocatalytique du glycérol brut

En contrôlant le courant appliqué, le pH de l'électrolyte, la concentration en glycérol et la durée de fonctionnement, et en sélectionnant les catalyseurs appropriés (électrodes), il est possible d'orienter les réactions d'oxydo-réduction et de favoriser la formation de produits ciblés. Des tests préliminaires ont été réalisés avec du glycérol pur pour identifier la meilleure gamme de conditions de fonctionnement et de distributions de produits en termes de pH, de densité de courant, de matériaux des électrodes, de durée de fonctionnement et de connaissance des mécanismes. Il a été constaté que le pH et le potentiel d'électrode au cours d'une électrolyse contrôlée à long terme ont des effets significatifs sur la conversion électrochimique (réaction redox) du glycérol. Comme on pouvait s'y attendre, la conversion du glycérol a été proportionnelle au temps d'électrolyse et les meilleurs résultats (formation du produit) ont été atteints dans des conditions acides plutôt que dans des conditions neutres ou basiques. L'acétol, le DHA et le glycidol en tant que produits non acides, l'acide formique, l'acide acétique et l'acide lactique en tant que produits acides constituent les principaux produits de ces expériences en solution acide dans la plage de potentiel appliquée «région de l'oxygène».

Le platine favorise l'oxydation du groupe alcool primaire, ce qui entraîne la formation de composés carboxylates. Si les potentiels sont plus élevés (par exemple dans la région oxygène), le clivage de la liaison C-C devient dominant et les espèces contenant C_1 et C_2 sont produites. Cependant, les taux élevés de conversion du glycérol et de sa cinétique ne

correspondent qu'à une faible quantité de rendement en produit. En conséquence à ceci, le glycérol pourrait avoir été converti en d'autres produits secondaires volatils tels que le CO_2 et qui ne pourraient pas être détectés dans la phase liquide du système. Ce résultat pourrait être dû au besoin d'énergie électrique élevé pour l'adsorption de la molécule OH^- sur la surface de l'électrode de Pt afin d'améliorer le processus électrochimique.

1.6 Conclusions

L'utilisation de ressources renouvelables pour produire des produits chimiques et des carburants est en augmentation. Les matières organiques provenant des déchets et du dioxyde de carbone sont renouvelables et servent de sources alternatives pour la production de biocarburants et de produits biochimiques. Ces travaux présentent une étude portant sur la production de solvants et d'alcools à partir de matière organique par électrosynthèse microbienne en tant que nouvelle technologie émergente. L'utilisation d'un système assisté par l'électricité dans le métabolisme des microorganismes anaérobies (biocatalyseurs) a montré une amélioration de la production de solvants par rapport aux méthodes de fermentation conventionnelle. Les électrotrophes, des souches microbiennes capables d'accepter des électrons de la cathode pour réduire les sources de carbone organique en des substances biochimiques de valeur, présentent désormais un intérêt particulier. Le glucose, le glycérol et d'autres composés organiques pourraient être convertis en solvants organiques à haute valeur ajoutée tels que l'éthanol, le butanol, l'acétone et le propanediol.

Une série d'expériences a été réalisée pour étudier la production de biobutanol en tant que nouvelle technique applicable au biocarburant dans une cellule de bioélectrolyse (BEC). L'objectif de ce travail était de comprendre le mécanisme et le taux de production du biobutanol par bioélectrosynthèse (BES) en utilisant du glucose comme substrat. Quatre principaux facteurs à savoir le matériau des électrodes, la concentration du substrat, la température de fonctionnement et les tensions appliquées ont été étudiées en batch afin d'atteindre les conditions optimales pour la production du butanol par C. pasteurianum dans BEC. Le support P2 standard modifié (MP2) et le support minimal standard (SMM) ont été utilisés comme milieu de fermentation en mode de fonctionnement par lots. L'optimisation numérique en utilisant une méthodologie de surface de réponse (plan centrale composite) a été utilisée pour maximiser la

production de butanol dans ce cadre expérimental. La production maximale de butanol, de 13,31 g/L, a été obtenue en appliquant 1,32 V qui indique la pertinence de cette procédure. Les résultats ont montré qu'en opérant dans les conditions optimales dans le SMM, le butanol pourrait être amélioré de manière remarquable par les microorganismes électroactifs dans la chambre cathodique.

Le 1,3-propanediol (1,3-PD) est aujourd'hui largement utilisé dans l'industrie des polymères. Il est produit par fermentation. La technologie de synthèse électromicrobienne (ESM) a été utilisée pour améliorer la formation des produits à haute valeur ajoutée tels que le 1,3-PD. Ce procédé dépend de la forme oxydée et réduite du nicotinamide adénine dinucléotide (NADH et NAD^+) et des équilibres électroniques dans les réactions redox. En ce qui concerne ces travaux, une production de 7,4 g/L de 1,3-propanediol (1,3-PD) a été obtenue en utilisant ESM et *C. pasteurianum* dans un réacteur de type H discontinu, avec du glycérol pur et brut utilisés comme substrats organique (rapport C/N de 36,9 g/g).

La conversion électrocatalytique du glycérol a été réalisée dans un réacteur discontinu de 500 mL. Une expérience initiale utilisant 10 g/L et 30 g/L de glycérol pur a montré que l'électrode de platine pouvait générer des produits chimiques à valeur ajoutée. La synthèse du produit non acide a été étudiée et optimisée en utilisant le plan central composite. La production globale au cours des 12 heures d'exploitation a été considérée comme critère d'optimisation. De plus, trois variables ont été choisies notamment la concentration du glycérol, l'acidité et le courant appliqué. Cette méthode peut être utilisée pour éliminer les déchets et produire simultanément des produits à valeur ajoutée. Une forte oxydation du glycérol a été enregistrée à un courant appliqué élevé, mais en augmentant l'intensité du courant. La conversion du glycérol en dioxyde de carbone et en produits acides augmente. La conversion électrochimique du glycérol brut traité en divers composés à valeur ajoutée tels que le glycidol, l'acétol et le DHA a été réalisée dans des conditions très acides (pH d'environ 1,4).

1.7 Recommandations et travaux futurs

Cette étude porant sur l'électrolyse chimique et microbienne de déchets agro-industriels pour la production de composés à haute valeur ajoutée n'est qu'une première approche. Un travail experimental pluridisciplinaire doit être poursuivi pour :

1- Synthétiser de nouveaux catalyseurs en vue d'orienter les réactions vers les produits ciblés (minimiser autant que possible les réactions parasites). Par exemple, il est possible d'éviter la formation du CO_2 lors de la conversion électrochimique du glycérol en utilisant des électrodes appropriées telles que Bi-Pt (platine combiné au bismuthe) ou Ir-Pt (Iridium combiné au platine).

2- Utiliser des techniques de séparation moléculaire (par exemple par chromatographie) dans l'optique d'obtenir les produits chimiques purs après leur sortie du réacteur.

3- Modifier génétiquement les microorganismes pour augmenter le taux de production et augmenter la résistance aux conditions de fonctionnement.

4- Réaliser une étude faisabilité technico économique et tester le procédé (électrosynthèse microbienne et conversion électrochimique) à plus grande échelle en intégrant le bilan énergétique.

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Deuxième partie

ARTICLES

CHAPITRE 2

Microbial Electrosynthesis of Solvents and Alcoholic Biofuels from Nutrient Waste: A Review

L'électrosynthèse microbienne des solvants et des biocarburants alcooliques à partir des déchets nutritifs: revue de littérature

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2 Microbial Electrosynthesis of Solvents and Alcoholic Biofuels from Nutrient Waste: A Review

2.1 Abstract

Using renewable resources to produce chemicals and fuels is increasing. Organic materials from wastes and carbon dioxide are renewable and serve as alternative sources for the production of biofuels and biochemicals. This review provides a survey of solvents and alcohols production from organic matter by microbial electrosynthesis (MES) as new emerging technology. Usingthe electricity-assisted system in anaerobic microorganisms metabolism (biocatalysts) showed improvement in solvent production compared to conventional fermentation methods. Electrotrophs, the kind of microbial strains which can accept an electron from the cathode to reduce organic carbon source materials to valuable biochemicals is of interest nowadays. Glucose, glycerol, and other organic compounds could be converted into high value-added bio-solvents, such as ethanol, butanol, acetone, and propanediol. This review addresses the electricity-driven microbial synthesis of chemicals especially solvents and alcohols by reduction of multi-carbon substrates considering the characteristics and advantages of MES.

Keywords: Organic waste resources; biofuels; electroactive biocatalyst; electrolysis; alcohols and solvents.

Résumé

L'utilisation des ressources renouvelables pour produire des produits chimiques et des carburants est, de nos jours, éminemment sollicitée. Les matières organiques, issues de déchets et du dioxyde de carbone sont renouvelables et se pésentent comme sources alternatives aux productions de biocarburants et de produits biochimiques. Cette revue apporte une étude de la production de solvants et d'alcools à partir de la matière organique par électrosynthèse microbienne (ESM) en tant que technologie émergente. L'utilisation d'un système assisté par l'électricité dans le métabolisme des microorganismes anaérobies (biocatalyseurs) a montré une amélioration de la production des solvants par rapport aux méthodes de fermentation classiques. À la lumière des intérêts actuels, les électrotrophes sont des souches microbiennes qui peuvent accepter un électron provenant de la cathode pour ensuite réduire les matières organiques (sources de carbone) en des produits biochimiques intéressants. En effet, le glucose, le glycérol et d'autres composés organiques pourraient être convertis en bio-solvants à valeur ajoutée notamment l'éthanol, le butanol, l'acétone et le propanediol. Par ailleurs, cette revue aborde la synthèse microbienne de produits chimiques, en particulier les solvants et les alcools, grâce à la réduction des substrats multi-carbone. Elle met également en évidence les avantages de l'ESM.

Highlights:

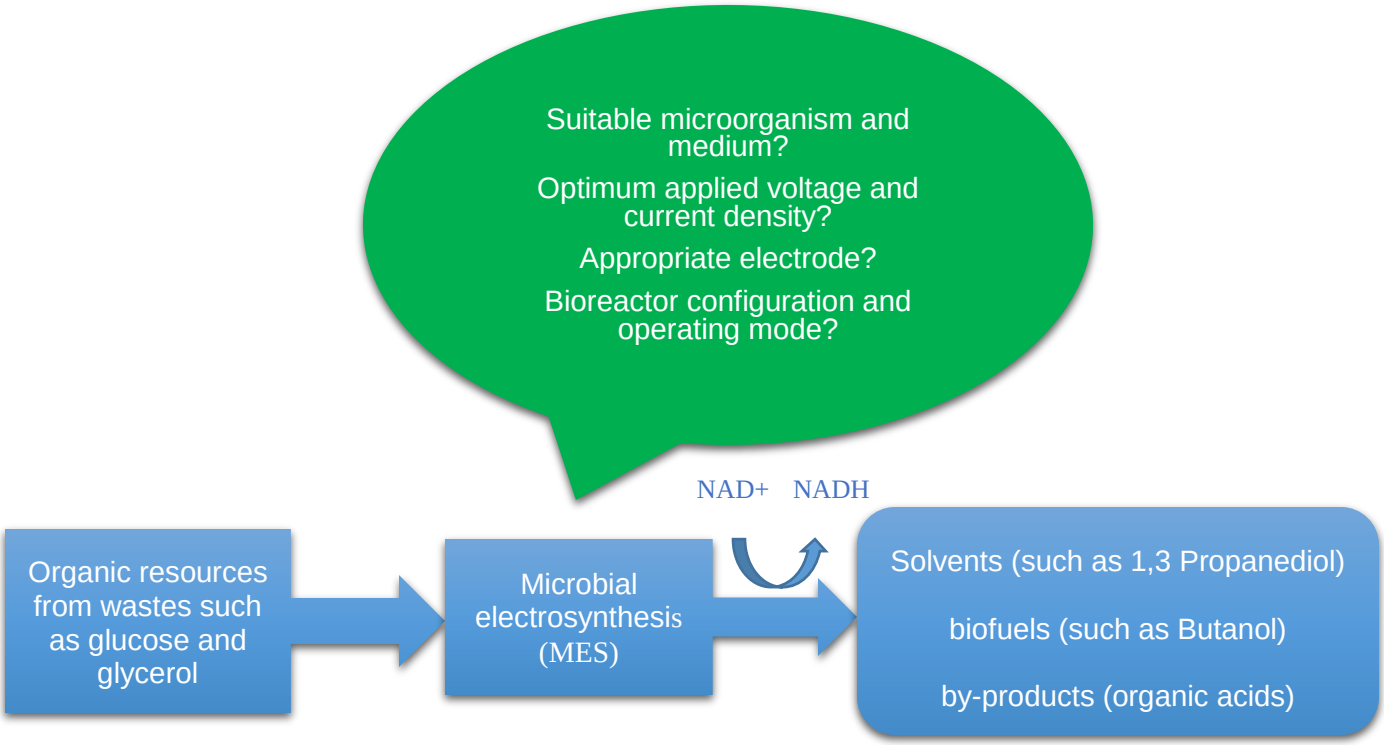
- Fermentation of carbon resource materials into solvents and biofuels by the aid of the electrolysis cell is reviewed.
- Influence of significant factors on the electro-bioreactor operation and electron transfer mechanism are investigated.
- Types of microorganisms and bioreactors are introduced.
- Future perspectives and challenges are outlined.

Main chemical compounds studied in this article:

Butanol (PubChem CID: 263); Acetone (PubChem CID: 180); Ethanol (PubChem CID: 702); 1,3-Propanediol (PubChem CID: 10442); Glucose (PubChem CID: 5793); Glycerol (PubChem CID: 753)

Abbreviations: ABE, Acetone-Butanol-Ethanol; emf, electromotive force; E-BCs, Electrobiocommodities; HVAP, High value-added product; MES, Microbial electrosynthesis; MEC, Microbial electrolysis cell; MFC, Microbial fuel cell; EF, Electro-fermentation; WW, Wastewater; PEMs, polymer electrolyte membranes.

Graphical abstract



2.2 Introduction

Nowadays, bio-fuel production from biomass or waste material is being performed around the world in the laboratories or pilot plants. Because of the abundance of organics in wastewater, it is considered as one of the most important alternatives to fossil derived and crude oil derivatives. Despite the production and use of renewable energy (hydro, wind, etc.), large quantities of oil (heavy oils, gasoline, diesel, natural gas, etc.) are still consumed. The use of renewable energy, combined with bioenergy, allows us to be more independent in energy matters and fight climate change. Among bioenergy, bio-alcohols as high value-added products from agro-industrial waste as an organic carbon source can be generated by fermentation of waste during electrolysis.

Electrobiocommodities (E-BCs) are organic compounds or fuels produced by carbon dioxide using electrical energy [1]. Microbial reduction leads to more specific compound production and lowering the energy required [2]. Chemolithoautotrophs are microorganisms which gains energy by oxidizing inorganic compounds [3]. Photoautotroph obtains energy from light and produces a renewable source of energy using inorganic matter. The microorganism that derives electrons directly from the cathode is called electrotroph which can accept electrons to reduce substrates [4]. In this category, acetogenic bacteria could produce bio-organic compounds, such as acetate and ethanol by use of gas substrates, such as CO_2, CO, H_2, and N_2 [5]. On the other hand, heterotrophic microorganisms utilize organic compound as a carbon source by electrofermentation (using electron from the cathode as co-reducing equivalent) which is focused on the present review. In recent years, microbial electrosynthesis of different products, such as hydrogen, hydrocarbons, and alcohols has been explored and some aspects of MES have been reviewed. In this regard, dynamic model, optimization and process control,

operational parameters in MES processes, advances and future perspectives, basic principles, biocatalysts role in MECs, hydrogen production, wastewater treatment and energy recovery have been reviewed [6-13].

This paper reviews the microorganisms, substrates and products, electron transfer mechanism, processing conditions, and system configurations regarding MES, focusing on solvent (non-acidic) production.

2.2.1 Environmental benefits and evolution of MES

Apart from biochemicals production, MES can also be used for wastewater treatment and pollutant removal. MEC encompasses several new applications, such as microbial electrodialysis cell, microbial saline-wastewater electrolysis cell, microbial electrolysis desalination and chemical-production cell, and microbial reverse-electrodialysis electrolysis cell [7]. Wastewater is the source of environmental pollutants, but at the same time, it can be used as a renewable resource of water, electricity production in microbial fuel cells (MFCs) and producing commodity biochemicals and bioremediation (removal of pollutants). Resource recovery and reuse can be achieved by integrated MES processes and waste biorefineries. In addition, by a variety of reactions potentials in MESs, selective products could be achieved from waste streams [14]. Variety of biomass can be used in solvent fermentation, such as CO_2, syngas, amino acids, cellulose, apple pomace, wastewater sludge from palm oil processing, date-palm fruit spoilage, milk dust powder etc. [15]. Since MES does not require a large amount of water for processing biomaterials to biofuels, it can reduce the detrimental environmental impact of large quantities of biomass production [2].

The traditional fermentation method for using non-food biomass such as cellulose and hemicellulose in agricultural products and wastes have been developed at laboratory scale for butanol production. Utilizing agricultural waste material by hydrolyzing the hemicelluloses can extend the amount of raw material for acetone and butanol (AB) production [16]. Domestic, agricultural, and industrial wastewaters which contain organic matter must be treated before discharge to the environment. The microbial electrolysis cell is one of the promising technology for production of chemicals containing energy from wastes and it has improved over the past few years. If this technology is to achieve practical application for wastewater treatment will face numerous challenges such as, elaborating the degradation of complex compounds, and controlling microbial reactions in the bioreactors. [17]. For instance, Ellis et al. [18] performed a study on acetone, butanol, and ethanol (ABE) production using *Clostridium saccharoperbutylacetonicum* N1-4 using wastewater algae biomass. They performed batch fermentation with 10% algae as a feedstock. Likewise, They produced 2.74 g/L and 9.74 g/L of total ABE using pretreated algae and by adding xylanase, cellulose xylanase and cellulose enzymes to fermentation system, respectively. Logan and Rabaey [19] reported that although the complex structure of various wastes resources needs a diverse microbial community to degrade the organic materials, several companies currently are in a process of industrializing MECs for treatment of wastewaters and production of biochemical, caustic and hydrogen peroxide solutions.

Various types of waste can be used as feedstocks for producing value-added products, such as acetate, fermentable organics (glucose, galactose, mannose, xylose, pure glycerol, biodiesel by-product, glycerol, high-strength wastewater (industrial and food processing wastewater, winery wastewater, etc.), lignocellulosic biorefinery by-products, and fermentation effluent

(cellulose fermentation, corn stover, lignocellulose fermentation, crude glycerol fermentation, etc.) [20].

Several studies agree that the biobutanol is a biofuel with little or no impact on the food supply because it can be produced from cellulose and wood residues. In fact, when biobutanol is produced from organic plant matter, it has a neutral CO_2 balance [21, 22]. The main advantages of using organic matter (e.g. wastewater) compared to CO_2 are lower electron consumption, easier biocatalyst growth, and using current infrastructure for bioproduction [23]. The quality and stability of bioproducts are closely related to the type of biomass used for synthesis. In such a process (MES), bacteria catalyze reduction reactions of organic molecules (at the cathode) or carbon dioxide. These reactions are related to bacterial respiratory metabolism.

The trend of related publications in microbial electrolysis field shows that the first study was done on L-glutamic acid fermentation using electro-energizing method [24] and it has evolved from 1979 till present (from Scopus). Actually, the number of published papers has risen gradually over the years, and there is a peak in recent years. Regarding the subject area, environmental sciences, chemical engineering, chemistry, and energy have shown the highest contribution to MES.

2.2.2 Bio-platform chemicals production in a microbial electrolysis cell (MEC)

Bio-hydrogen and methane as a sustainable and clean fuel can be produced by cutting edge MEC technology using different wastewaters, CO_2, organic matter, and biomaterials by applying hydrogenotrophic microorganisms. In recent years, the pilot-scale of the latter process has been erected, albeit some challenges remain [1, 12, 25]. Acetogenic bacteria can reduce CO_2 to

acetate which is a precursor to solvents. Acetogens, such as *Sporomusa ovata*, *Clostridium ljungdahlii*, *Clostridium aceticum*, *Moorella thermoacetica,* and *Acetobacterium woodii* can produce value-added products by fixing waste greenhouse gases. They use acetyl Co-A pathway to acetate production, however genetic mutations, metabolic engineering, and bioelectrochemical synthesis can lead to more solvent production, such as ethanol, isopropanol, and butanol [5]. Special products can be produced by the engineered microorganism. Compared to conventional ABE fermentation, cellulolytic and acetogenic *Clostridium* are able to be engineered to transform cellulose, CO_2 and H_2, to n-butanol with high yield [26]. It has been reported that CO_2 can be converted to higher alcohols, such as isobutanol and 3-methyl-1-butanol (3MB) in MEC with *Ralstonia eutropha* H16 [27]. Hydrogen peroxide is another bioproduct of MES which is formed based on the oxidation of organic waste at the anode and combined with the reduction of oxygen at cathode [28]. In the patent published by Lovley and Nevin [29], the final products of MES included acetate, butanol, propanol, ethanol, 2-oxobutyrate and formate by use of mixed culture.

Liquid products, such as acids [30], acetone, butanol, 1,3-propanediol (PD) (by reduction) and ethanol (by oxidation/reduction) are the most important chemical products which can be produced by this technology using organic resources. Applications of 1,3-propanediol in the polymer industry, solvents, cosmetics, lubricants and antifreeze have further raised its interest [31]. Annually, 200000 tons of 1,3-propanediol is produced due to its wide usage in industry. It can be biologically produced using glucose and glycerol as substrate [32]. The most common biofuel around the world is bioethanol. As it is an oxygenated fuel, it results in NO_x emission reduction. Butanol or ethyl alcohol comprises four carbons in its structure (C_4H_9OH) and has four isomers. In recent years, biobutanol has been considered as alternative biofuel rather than bioethanol due to its properties, such as higher energy content and flash point and lower

volatility, and vapor pressure. Biobutanol is known as second generation biofuel which can be mixed with gasoline in higher portion than ethanol [15]. Biobutanol also has disadvantages, such as lower octane number than bioethanol and its toxicity [33]. Butanol is also used as raw material for dye, buthyl acetate, phenol formaldehyde resin, nitro enamel, and plastificator [34].

2.3 Microorganisms

Clostridium species produce alcohols and solvents in appropriate media. Different microorganisms and media which have been used for electricity-assisted fermentations for solvents production are tabulated in Table 2.1. Solventogenic *Clostridia* (Gram-positive bacteria) produce butanol, ethanol, and acetone. By utilizing mono and polysaccharides, these species can produce alcohols which require high redox potential (additional power). Solventogenic *Clostridia* metabolize under anaerobic conditions which are able to produce a wide range of primary metabolites, such as butanol, by means of degradation of carbohydrates [35]. Fig 2.1 shows the general metabolic pathway for microbial electrosynthesis by *Clostridia* species using different substrates.

The microbial reduction reactions take place at the cathode for producing chemicals which have been developed in recent years include microorganisms that act as biocatalysts in electrochemical reactions [9]. Glucose and /glycerol are the main substrates for the synthesis of alcohols by MES. They can be obtained from wastewaters or biomass. The suitable medium is also required to supply nutrients and chemicals to provide the best conditions for microbial activities. The different feedstock can be used for bioprocess by *Clostridia,* such as sucrose and starch, lignocellulosic biomass, glycerol, algal biomaterial, and syngas. Each feedstock has its pros and cons. For instance, in case of butanol production, butanol productivity is higher by

using sucrose, but there is a limitation in butanol production using glycerol by *Clostridia* strain except for *C. pasteurianum* which can produce butanol even using immobilized cell [36, 37]. One of the constraints of butanol production by mentioned bacteria is intolerance of *Clostridia* to butanol (solvent toxicity), and typically 12-16 g/L of butanol are required to inhibit the cell growth during the fermentation. Modified strains can tolerate more than the regular concentration of butanol, such as *Clostridium acetobutylicum* JB200 which produced butanol up to 21 g/L [15]. *C. beijerinckii*, *C. acetobutylicum, C. saccharobutylicum*, and *C. pasteurianum* are the most suitable microorganisms to produce biobutanol and solvents. Recent studies revealed that by utilizing *C. pasteurianum,* inoculum age of 16 h, initial pH of 6.8, and temperature of 30 ^{o}C with 50 g/l glycerol as a substrate for conventional fermentation, and 118.33 g/l of glucose concentration, temperature of 33.51 ^{o}C, and 1.32 of applied voltage for MES of butanol production are the optimum conditions. Also, by using *C. acetobutylicum,* glucose concentration of 50 g/L, butyric acid concentration of 8.7 g/L, and C/N ratio of 65 (optimum condition) led to 13.87 g/L butanol production [15, 38-40]. Apart from *Clostridia*, the specific engineered *E.coli* strains can also produce butanol and branched-chained alcohols [41].

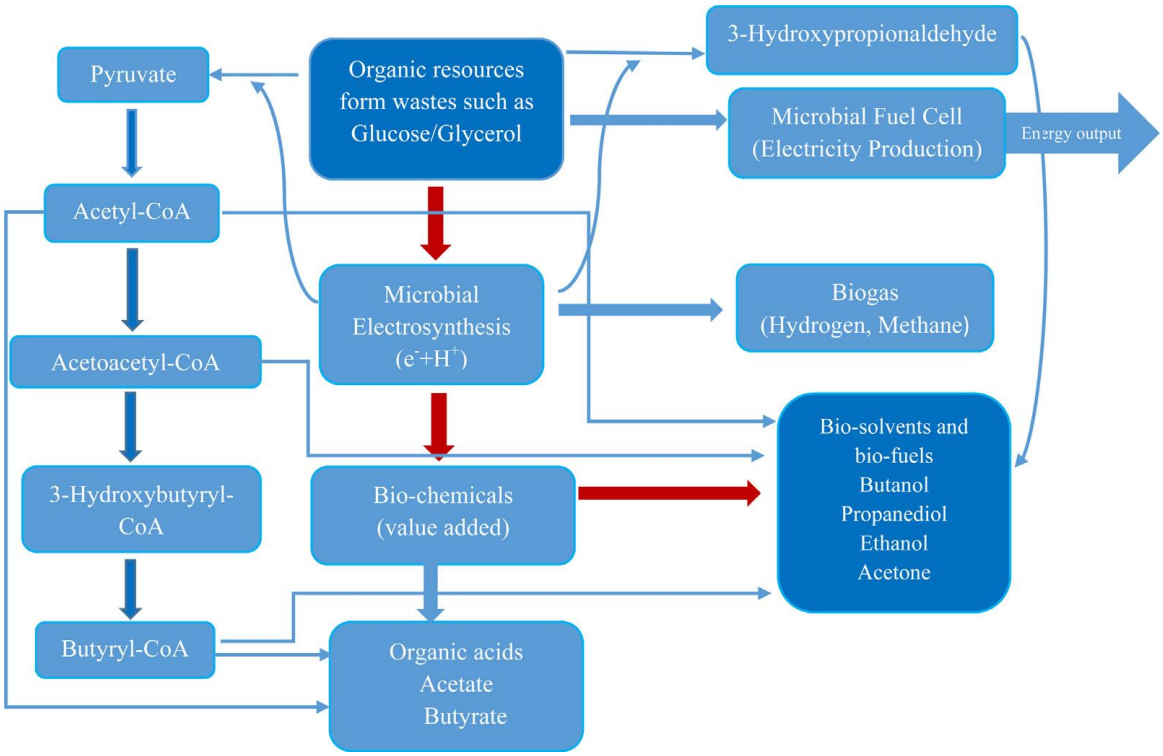


Figure 2-1 Simplified integrated biological mechanism and electricity production/consumption by microbial electrolysis for production of value-added products such as acids, solvents, alcohols, and biofuels. The red axes show the route of this study, although the waste can be used in a microbial fuel cell or, for biogas production.

Table 2-1 Microbial electrosynthesis of solvents and biofuels.

Reactor type	Electrode material	Voltage (V)	T (ºC)	pH	Time	Microorganism	Medium	Membrane	Products	Reference
Bottle batch fermentation	Platinum wire as the working electrode and a carbon rod as the counter electrode	+0.5 to 0.5 V vs Ag/AgCl),	-	5.8-6.8	96 h	*Clostridium sporogenes* BE01	Hydrolyzed rice straw+ $CaCO_3$ and yeast extract TGY medium for inoculum generation.	-	Butanol, ethanol Fatty acids hydrogen	[105]
A two compartment electrolytic cell	Anode: Pt wire Cathode: graphite fiber, Graphite felt, neutral red immobilized graphite	4V external energy supply	30	5.5	4 days	*Strains CDBT*	Peptone Yeast Extract Nitrogen (PYN) media with glucose and Pretreated Lignocellulosic Biomass Hydrolysate	Porcelian membrane and cellulose acetate film	Ethanol	[106]
Two-compartment cell separated chamber	Modified electrodes (Immobilized carbon felt): a working electrode, Pt foil: counter electrode	_600 mV vs. Ag/AgCl	Amb	7.65	8 hrs	Alcohol dehydrogenase enzymes	Butyraldehyde +TRIS-HCl buffer		Butanol	[107]
H-type reactor with a dual-chamber, batch	Graphite felt electrodes (both)	-700 mV vs. Ag/AgCl	35	6.0	40 h	*Clostridium beijerinckii* IB4	P2 medium with electron carries and glucose	Cation exchange membrane	ABE production, butanol main product	[54]

fermentation										
two compartment type of electrochemical cell	-	-2.5 V applied to the cathode chamber	35	7.4	60 h	*Clostridium acetobutylicum* ATCC 4259	CAB medium with buffer solution and electron carriers	Cytoplasmic membrane	Butanol acetone	[84]
Lamellar-type reactor consisting of two end plates and three paired anode-cathode compartments	Graphite	-1.505 V vs Ag/AgCl	-	5.5	9 weeks	*Citrobacter Pectinatus Clostridium*	Glycerol +additives	Cation-exchange membrane	Solvents and acids	[108]
Construction Dual-chamber, H-type borosilicate reactors fed-batch mode	Graphite felt	−1.1 V vs. SHE	37	7.3-6.6	Up to 70 days	Mixed microbial consortium *Clostridiaceaen Veillonellaceae Lactobacillaceae*	Phosphate-buffered mineral medium+glycerol	Cation exchange membrane	1,3-Propanediol (1,3-PDO) as the main product	[55]
Two compartments batch and fed-batch system	Graphite plates	−0.9 V versus SHE	20-25	5.5	3 h	*Citrobacter Pectinatus Clostridium*	M9 medium+glycerol	Cation exchange membranes	1,3-Propanediol (1,3-PDO)	[31]
H-Type reactor, continuous mode	Unpolished graphite	-600 mV Vs Ag/Agcl		6.7-7	5-13 days	*Sporomusa, Clostridium, Geobacter, Morella*	DSMZ Medium with CO_2 injection	Proton-selective Nafion 117	Acetate;butanol; 2-oxobutyrate; proponal; ethanol; and formate	[29]
Dual-chambered, H-type	-	anode electrode was poised at 0.24 V vs Ag/AgCl	30	6	5-10 days	Exoelectrogen *Geobacter sulfurreducens* and the bacterium *Clostridium cellobioparum*	GS2 medium with glycerol	Bacterial cytoplasmic membrane	Hydrogen, ethanol and 1,3- propanediol	[109]

Two-compartment reactor	The working Electrode: carbon clothe The counter electrode: platinum mesh	0.2 V vs Ag/AgCl	30	opt: 6.0	48 h	*Enterobacter aerogenes* NBRC 12010	The basal medium, glycerol wastes, thionine, and phosphate buffer	Cation-exchange membrane	H_2 and ethanol	[110]
Flat plate reactor (pH controller)	Graphite felt both electrodes	-550 mV vs NHE	30	5.5-6.0	Max 22 days	Acetate-reducing inoculum (from anaerobic sludge blanket)	-	Monovalent selective anion-exchange membrane	Ethanol (main) methane butyrate	[58]
H-type dual chamber	Cathode: graphite felt, Anode: Pt plate	-0.16 V vs Ag/AgCl	37	6.5	50 h	*Clostridium pasteurianum*	MP2 with glucose and glycerol	Nafion 117 cation exchange	Butanol 1,3-propanediol	[56]
Two-chambered H-type	Titanium rod as anode and carbon graphite as a cathode	-0.8 V versus SHE	34-36	4.63 - 6.45	35 days	Species of the genus *Clostridium*+ carboxydotrophic mixed culture	CO_2+ organic matter-free media similar to ATCC175420	Cationic exchange membrane	Ethanol butanol acetate butyrate	[62]
Two-chambered H-type	Graphit felt and stainless-steel	1.32 applied voltage as optimum	33.5 opt	6.7	48-72 hr	*C. pasteurianum*	Standard modified P2 medium (MP2) and standard minimal medium (SMM)	Nafion 117 cation exchange	Solvents, acids, mainly butanol	[39]

2.3.1 Microorganisms and electric charges

MES performance is affected by some important parameters, such as microbial physiology, materials of electrodes, type of electrolytes and substrates, and redox potential [8]. Exoelectrogens are able to transfer electrons to the anode (current generation), on the other hand, endoelectrogens can accept electrons from the cathode to reduce substrate (current consumption) [42]. In MEC, the power is used to enhance the reaction kinetics. The electron transfer mechanism between external microorganisms and the electrode can be made either directly or indirectly. The electron transfer is via direct contact between the electrode and the microorganisms via cytochrome C and other proteins from the bacterial membrane capable of transporting a fraction of the electric current. In an indirect process, the microorganism-electrode interaction takes place via mediators which are capable of shuttle electrons between the electrode and the microorganisms. Recent studies have shown that several acetogenic bacteria are able to consume the electrons from the cathode electrode, wherein the process could be coupled to the reduction of CO_2 to produce acetates [10]. The direct process of electron transfer within the bacterial cell requires the attachment of a bacterial biofilm on the surface of the electrode. This bacterial development can cause a bottleneck on the surface of the electrode and limit the treatment and recovery of waste. To solve this, it is possible to use shuttles (or carrier) for the transfer of electrons from the bacterial cell to the electrode. Direct electron transfer involves periplasmic and outer membrane complexes. A decahaem cytochrome (on the surface membrane) can donate electrons. Nanowires are the other mechanism of direct electron transfer between microorganism and electrodes. Building blocks, like formate or acetate, can also be used as an intermediary microorganism which is utilized by other microorganisms to produce more complex chemicals. For instance, *Shewanella* spp. produces flavins as electron mediators which play the intermediate chemical role to produce their own intermediates that subsequently react with the other cells [10]. Electron mediators (which

can rise the NADH pool) might be dependent on the species. Some electron mediators, like NR, improved butanol production in the acetone–butanol–ethanol (ABE) fermentation [43,57].

Although, the microbial cell envelope comprises an electrically non-conductive structure such as peptidoglycan, a cytoplasmic membrane (in cell envelope) of microorganism can transfer electrons. Extracellular electron transfer (EET) can be defined as the transmission of electrons from redox carriers (in the cytoplasmic membrane) to extracellular minerals and vice versa. Through c-type cytochromes and microbial nanowires, the microorganism is capable to exchange electrons. This kind of bacteria can be used in biofuel production and bioremediation [44].

Even though there are a lot of strains which are able to transport electrons outside the cells, transfer of electrons to the electrodes are limited owing to losses. Thus, performing research on the extracellular electron transfer mechanisms seems to be necessary in order to minimize losses. Understanding the mechanism of electron transfer between bacterial cell, finding the most electroactive bacteria, and exploring the most suitable and efficient electron mediator for each unique system are among the solutions to enhance the efficiency of value-added production in MEC.

2.3.2 Media and *Clostridia* species

It has been demonstrated that chemicals, such as K_2HPO_4, NaCl, and $MgSO_4$ have a significant impact on the production of biobutanol by *Clostridium* species [45, 46]. Moon et al. [47] used MP2 medium containing 20 g of glycerol, 100 mM of 2-(N-morpholino) ethanesulfonic acid (MES) to prevent over-acidification at 37^{o}C and adjusted 6.5 pH for production of chemicals, such as butanol and PD. They showed that the optimum media for producing butanol from glycerol by *C.*

pasteurianum contains (0.06 g/L of $FeSO_4.7H_2O$, 7.35 g/L of $(NH_4)_2SO_4$, and 5.08 g/L of yeast extract) as major components, but for 1,3-PDO production, the optimum values are: 0 g/L of $FeSO_4.7H_2O$, 0 g/L of $(NH_4)_2SO_4$, and 8.0 g/L of yeast extract. Basal medium is also used to produce butanol containing glucose [48]. In addition, *Clostridium* growth medium (CGM) is used to produce alcohols from *Clostridia*. Moreover, it has been reported that *C. pasteurianum* DSM 525 grows in the standard minimal medium at pH 7.0 [49]. As known traditionally, *Clostridium acetobutylicum* produces ABE, a mixture of acetone, butanol, and ethanol. *Clostridium beijerinckii* can also produce IBE, isopropanol, butanol and ethanol [50]. *Clostridium acetobutylicum* can produce butanol under a suitable condition in P2 medium and date fruit [51]. Also, it has been concluded that glucose is the most favorable organic matter for butanol production. Chen et al. [52] achieved optimum butanol production by use of *Clostridium beijerinckii* NRRL B592 from butyrate and sucrose in a series of batch fermenters. Peptone yeast extract-glucose (PYG) medium was used under the anaerobic condition for the experiments. Moreover, it has been reported that acetone-butanol and isopropanol could be produced by these bacteria [50]. In this case, ABE fermentation goes towards ABI fermentation in which acetone converts to isopropanol. Besides, non-pathogenic acetogens, such as *Clostridium autoethanogenum, Clostridium ljungdahlii* and *Clostridium ragsdalei* are also able to produce 2,3-butane diol (2,3BD). *Saccharomyces cerevisiae* is also used to form isobutanol and butanol. Genes of *Clostridium acetobutylicum* can also produce isopropanol [5]. Interestingly, among the anaerobic bacteria, *Clostridium acetobutylicum* YM1 is a new strain which can produce butanol from organic matter under aerobic conditions [55]. Actually, the growth profile of bacteria and solvent production were similar under both aerobic and anaerobic conditions. Some *Clostridia* species are suitable for solvent production and some of them are appropriate for organic acid production, thus selection of microorganism strains and proper media (such as P2 media) and substrate leads to the best results.

2.4 Bioreactor and process concept

As can be seen from Fig. 2.2, cathode chamber can be filled by the substrate and appropriate solution for growing microorganism and in case of anaerobic inoculation for removing residual oxygen, the reactor should be purged with a neutral gas, such as nitrogen/argon. At the anode compartment, the abiotic or biotic reaction may take place. In case of microbial electron production, the gram-negative bacteria can be used but alternatively, for abiotic reactions, anode chamber can be filled with phosphate-buffered saline, phosphate-buffered mineral medium, or hexacyanoferrate solution [$K_4(Fe(CN)_6.3H_2O$] [54- 58].

Different types of reactors with electrodes have been mentioned in the literature for MES. The common configuration is H-type batch bio-reactor equipped with cation exchange membrane in different sizes. Table 2.1 shows the types of reactors, electrode types, microorganism, membrane type, source (medium), and type of produced chemicals and poised voltage. The process can be performed in batch, fed-batch and continuous mode. A single cell with two anodes, multilayered anodes between two cathodes, porous anode combined with membrane, tubular reactor with the membrane, series of tubular MECs, MEC stainless steel wall as a cathode, MEC with hollow fibre membrane, and fluidized bed bioreactor with anode granular was also suggested for MES [13]. Two cells in parallelepiped shape, having a capacity of micro-scale to pilot scale was constructed by Krieg et al [59]. The reactors are compartmentalized by means of a cation exchange membrane (MEC) and consisted of two electrodes. The anode is made of graphite (Gr), and the cathode is metal of stainless steel/carbon with different inter-electrode distance. Such expanded metallic electrodes provided mechanical strength and high surface area (electrode-electrolyte). Wastewater/synthetic media were used as the substrates in the anode compartment. The treated effluent will then be transferred to the cathode compartment to be used as a culture medium. The cathode compartment works under anaerobic conditions. Various parameters affect reactor

operation, such as voltage across the electrodes, C/N ratio in the culture medium, processing time, type, and concentration of substrate, etc. Other parameters, such as the type of electrodes, active surface electrodes and the type of proton exchange membrane (Nafion) are also important.

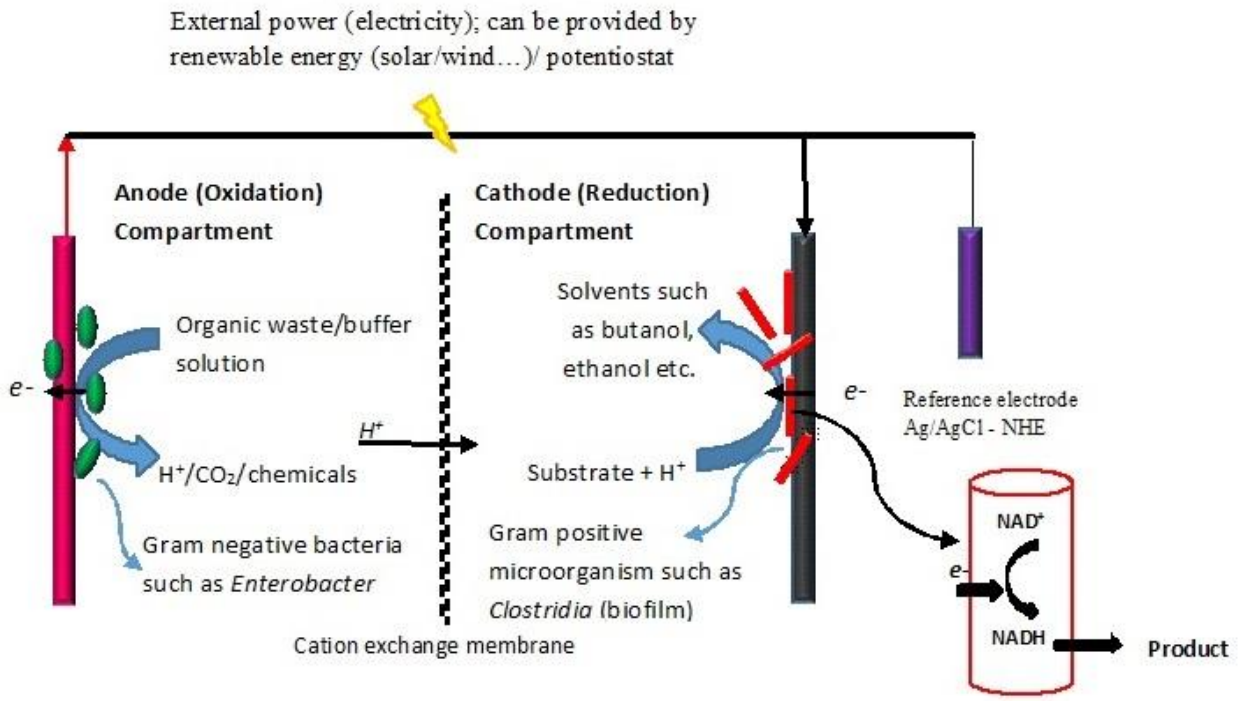


Figure 2-2 Schematic of electro-microbial Cell comprises two chambers. Anode reactions can be abiotic/biofilm reaction. If wastewater is used in the anode, the effluent may be used for nutrient recovery/process or drinking water. In cathode chamber substrate including CO_2/glucose/glycerol plus suitable synthetic medium /real wastewater. In the anode section, chemicals like ethanol and acetate can be produced. Each chamber should be stirred or recirculate to enhance efficiency.

To commercialize MES, it is important to realize the reactor concept, its components, and type of reactions. Several components affect reactor performance: electrode materials, separators of anode and cathode (to hold redox gradient), and the distance between electrodes. In terms of reactor types, three major configurations have been used: two-chambers/separated reactors, single chamber/ non-separated reactors, and packed and fluidized bed reactors.

The biocatalyst types and the source of reducing power result in flexible technology which is able to adopt in various processes. Different bioelectrochemical systems can be used to achieve final products, such as bio-anode in association with the chemical cathode, bio-anode along with bio-cathode, the chemical anode in a combination of biocathode, chemical/bio anode combined with cathode which reduces mediators [10].

In terms of fermentation process strategies, various efforts have been performed for cost reduction and optimization of the targeted product, such as butanol in ABE fermentation (non- electricity-assisted). In batch fermentation, using co-culture mediated fermentation leads to higher production from starch. The fed-batch operation is one of the desired commercialization processes due to the higher concentration and productivity of the final product. Continuous fermentation also reduces the time lag phase in comparison with the mentioned operational modes, but cell wash-out is a problem in this process which has an adverse impact on productivity. Fortunately, this issue can be solved by high cell density cultivation [36]. As a result, for laboratory investigation of solvent production in MEC, dual-chamber bioreactor equipped with proton exchange membrane and three electrodes is recommended.

The whole process of the traditional fermentation could be included milling of biomass, grading sieve, mash preparation tank, sterilization tank, cooling, fermentation (using prepared microorganism in separate section), broth storage tank, continues distillation to separate raw products and waste mash (along with the absorption tower to absorb produced gases like hydrogen and carbon dioxide), and fractionation to obtain final products such as, acetone, ethanol, n-butanol [60]. The key production parameters which should be attended in MES process compared to the conventional process are product titer, production rate, coulombic efficiency, electron regaining, and energy balance. Volumetric product rate formation and product titers are still presently low. In order to the improvement of MES technology, significant process parameters,

techno-economic analysis and life cycle concerns, must be studied [61]. Therefore, MEC processes need an amount of electrical energy, which it effects on the fermentation process, perhaps pretreatment and downstream sections.

By converting waste into value-added bioproducts in MECs, the system will be energy-positive and carbon-negative. Costs will be reduced, and performance of the MEC will be enhanced, if the reactor types and materials improved [7, 20]. Re-designing the bioreactor considering electrode and membrane costs, availability of nutrient waste, efficiency enhancement by optimization techniques and energy balance, and studying in depth of biotic treatment of wastewater in anode chamber and biosynthesis in cathode compartment simultaneously, would be the future research gates in reactor configuration.

2.4.1 The cathode and anode compartments

In most MES, (as can be seen from Fig. 2.2) on the anode surface, the hydrogen ion is produced and goes toward the cathode through exchange membrane and in the cathode chamber. Microorganisms utilize electrons and hydrogen ions along with substrate in reduction reaction to produce chemicals (electrodes are connected to electrical source). In the anodic cell, the simple reaction can take place, such as oxidation of water without any living strain (abiotic) or oxidation of wastewater using microorganisms. The latter could help reducing anode overpotential in MES [61]. At the anode, under abiotic conditions, water can be decomposed to protons and oxygen in the anode half reaction and the protons pass through the permeable membrane. At the cathode, half reaction includes conversion of carbon sources to carbon-bearing compounds by the biological film (bacterium which accepts electrons) provided on cathode [29]. Sugars mostly are used as a substrate in bioelectrochemical systems. Sadhukhan et al. 2016 showed the route of lignocellulose

fractionation to chemical production by MES fermentation. Glucose is converted to carbon dioxide/acetate, hydrogen species and electron at anode microbially, and glucose can be reduced to butanol, propionate, and glutamate in the anaerobic cathode. Carbon dioxide from the anode, on the other hand, can be circulated to generate biochemicals at the cathode. Thus, solvents could be produced from an organic source in the cathode compartment (by reduction), while in the anode compartment, the abiotic or biotic oxidation takes place.

2.4.2 pH Effect

Generally, the pH range between 6 to7 (as can be seen from table 2.1) is favorable for alcohol production in microbial synthesis depending on the product, substrate, and type of microorganism. Some experiments were performed under constant pH [31] by adding H_2SO_4 and pH controlling, but some of them were performed without controlling pH to investigate the pH change during bioreactions [62]. According to Moon et al. 2011, butanol production from sugars using *Clostridia* species was affected by pH, but *C. pasteurianum* was not affected by pH using glycerol as substrate. In a study by Dabrock et al. [49] (solvent production by *Clostridium* species), while pH increased from 5 to 7, glucose consumption, butyrate and acetate production increased, but ethanol and butanol production remained constant. Two-stage pH control in solvent fermentation using glucose and *C. acetobutylicum* XY16 showed that the duration of fermentation decreased and productivity, yield and concentration of products (ABE) increased. A study of MES in the pH range from 4.3 to 6.0 showed that ABE production (by *Clostridium* acetobutylicum XY16) was influenced by pH, nor lower neither higher values are suitable, but at higher pH, the organic acids production improved [63]. Moreover, it has been mentioned that organic acids re-utilize solvents at higher pH [64].

2.4.3 Electrodes and applied potential

Several factors can affect the performance of MES, such as specific surface area, biocompatibility, electron exchange between electrode and microbes, and conductivity. It has been recommended that a metallic electrode with coated carbon is the best choice for MES due to higher conductivity and resistance of metals, such as Pt and SS along with high biocompatibility of carbon-based materials. Electrode material selection depends on bacteria and reactions which take place in the cell [65]. Because fermentation time depends on carbon source consumption [66] and biofilm stability in MES, the material of the electrode has a significant effect on MES performance. There are two kinds of electrode systems in MES. The two-electrode system contains a working electrode (the main product is produced in this part), and a counter electrode (counter reaction took place in this part). In the mentioned system, the potential cannot be measured and controlled. Three-electrode system comprises a reference electrode which enables to set potential in working electrode compartment. Working and counter electrode can be anode or cathode according to reactions and targeted products. Krieg et al. [59] suggested an economical and stable 3-dimensional electrode to maximize efficiency in large scale bioelectrochemical systems. Pons et al. [67] revealed that biofilms structures influence current which is provided by cathodes. They proposed a dense layer of small and uniform colonies of a microorganism to achieve the best results. Stainless steel and nickel sheets were also used as cathode materials for hydrogen production in MECs. Copper and stainless steel are not suitable for anode material due to corrosion and toxicity, but graphite fibre brush is a promising material for anodic reactions [68]. In research conducted by lovley and Nevin [29], electrodes (cathode and anode) were selected from the following materials: carbon-based materials, such as carbon paper, carbon cloth, carbon felt, carbon wool, carbon foam, graphite or porous graphite, powder of graphite, graphene, nanotubes from carbon, electrospun carbon fiber, and the following metals, such as platinum,

palladium, titanium, gold, silver, nickel, copper, tin, iron, cobalt, tungsten,, stainless steel (SS) and conductive polymer. Gregory et al. [69] proved that graphite electrodes may assist as an electron donor directly for anaerobic respiration. This property can have a few environmental benefits, such as bioremediation of groundwater.

The applied voltage to MES is equal to theoretical voltage plus overpotential (the excess required potential due to resistance) which includes Ohmic overpotential (ion transfer between electrodes), anode and cathode overpotential (due to metabolism and activation losses) and concentration overpotential (increasing cathode chamber pH). To reduce overpotential, Ki et al. [70] suggested sparging CO_2 in the cathode chamber and using improved materials as cathodes. In MEC, the energy received by cathode electron donor should be maximized and energy utilization by strains must be minimized to optimize biocathode function. Direct electron transfer reactions, c-type cytochromes along with hydrogenases, mediated electron transfer reactions (natural redox mediators) are recognized mechanisms for biocathodic reactions [71].

Gibb's free energy which can be converted to standard potential E^o plays the important role for calculating the voltage needed or produced in an MES reaction, for instance, ethanol and hydrogen consuming electricity while methane and hydrogen peroxide are thermodynamically favorable. Overpotential (energy lost at the electrode) and the coulombic efficiency (produced electrons end up in the targeted product) indicates the performance of electrodes. In cathodic reactions, when the cathode potential is lower than anode potential, the energy (as electricity form) is required to produce final products, such as solvents and biofuels [72]. For instance, if oxidation of glucose to HCO_3^- (in anode chamber E^o= -0.41 V) is coupled with butanol production at cathode by reduction of HCO_3^- (E^o= -0.30 V), the cell voltage will be +0.11 V, so theoretically butanol can be produced without any external source of energy without considering overpotential. Assume that

at the anode, water is electron donor (E^o=0.82 V), consequently, the cell potential is -1.12 V, thus the residual energy as electricity is required. As a result, the oxidation of organic matter reduces energy consumption in MEC [10].

The theoretical cell voltage in MEC is calculated from the Gibbs free energy of the overall reaction as follows [17, 73]:

Equation 2-1 $$emf = \frac{-\Delta G}{nF}$$

where emf is the electromotive force (potential difference between electrodes) in Volt, ΔG is Gibbs free energy in J/mol, n is the number of electrons participate in the overall reaction in mol, and F is Faraday's constant F=96485.3 C/mol. Since ΔG can be calculated from the following equation:

Equation 2-2 $$\Delta G = \Delta G^0 + RTln(\pi)$$

where ΔG^0 is defined as Gibbs free energy at standard condition (298.15 K, 1 bar pressure, and 1 M concentration for all species), R is the universal gas constant, T (K) is the absolute temperature, and π (dimensionless) is the reaction quotient (activities of the products divided by those of the reactants), the emf can be calculated as:

Equation 2-3 $$emf = emf^0 - \frac{RT}{nF}\ln(\pi)$$

and the cell emf is calculated as follows:

Equation 2-4 $$emf = emf_{cat} - emf_{an}.$$

Electrolyte ohmic loss (in V) depends on the distance between electrodes, current density, and conductivity of fluid as the following equation:

Equation 2-5 $$\Delta V_{\Omega} = \frac{dj}{\sigma}$$

where *d* is the distance between cathode and anode (in m), *j* is current density (in A/m^2), and σ is conductivity (in S/m).

Current density is another important parameter which affects the performance of MES which operate at a set potential. Higher current density leads to the significant production rate of bio-products. By increasing the cathode surface area, the higher current density can be achieved by the use of porous materials [8]. Some studies were done under constant poised voltage and some of them preferred to test variable one. The research [74] showed that for wastewater treatment via bioelectrochemical systems by sludge, COD removal efficiency enhanced by applying voltage lower than 0.8 V, while it decreased when voltage went up to 1.0 and 2.0 V. Performance of polarized and non-polarized cathode examined for production of 1,3-propanediol from glycerol showed that consumption of substrate and production rate increased in polarized case. Hydrogen supply also had a positive effect on product formation but not as much as the polarized cathode system [31].

Generally, electrodes should have the following features: moderate-to-high conductivity, biological, chemical, and physical stability, economically worthwhile, and high specific surface area. For anode and cathode, carbon-based materials can be used and available with a wide range of costs from one thousand dollars to \$10-50 per 1 m^2 [13]. Carbon-based materials have been used broadly for electrodes. The conductivity of carbon-based electrodes are fairly low in comparison with metals, however, using metallic collectors can compensate this drawback. Metallic electrodes (such as titanium and stainless steel) seem to not be suitable due to their lower surface area and surface properties which result in less biofilm development. In a study, an electrode consisting a graphene sponge integrated with stainless steel (SS) current collector showed a good performance with acceptable cost (2 mm-thick graphene sponge costs \$4 /m^2) [13]. To compare carbon-based

electrodes, it is worth noting that carbon cloth or carbon fibre costs around the US $5-25 per 1 m^2, while graphite felt costs around the US $1.0-100.0 per 1 m^2. Also, in terms of a mass basis, carbon felt costs about US $37-46 / kg, the price of glassy carbon is the US $1660-4285 per metric ton, and graphite costs the US $ 1650-4650/Ton which show the wide range of prices for application in various systems [75].

By considering the costs and advantages of carbon-based materials it is recommended to utilize these materials as electrodes in MEC. Using graphite and carbon-based materials as electrodes have various benefits, such as suitable cost, higher surface area (due to porosity), and stability, but they have higher resistance and lower mechanical strength compared to metallic electrodes.

2.4.4 Membrane

As seen from Table 2.1, batch and fed-batch laboratory reactors equipped with membrane were used for MES. Moreover, graphite and carbon-based materials were used as electrodes. Cation exchange membrane is the most favorable separator and temperature between 30-37 ^oC is the most appropriate condition for microbial metabolism. The most suitable pH is around 6-7 (neutral pH) and also *Clostridium* species are very popular for solvent and alcohol production. Nafion-117 proton exchange membranes (chemically stabilized perfluorosulfonic acid (PFSA) polymer) can be selected as the most favorable membrane material for MES. The operation of MES depends on membrane selectivity and performance to separate anode and cathode compartments. As mentioned earlier, the most common type of membrane is the proton exchange membrane which allows the needed flow of protons through the membrane to supply the cathode section. New membrane material like forward osmosis membranes can increase the reactor performance for wastewater treatment in MEC [76]. Carbon cloth can also be used between cathode and anode

chambers. By using the membrane, produced gases are isolated in each compartment, the microbial section is separated from the other section, and it leads to prevention of contamination [77].

In terms of membrane structure, heterogeneous membranes have higher mechanical strength but have higher resistance which results in higher ohmic overpotential, homogenous membranes, on the other hand, are thinner and cause lower resistance [70]. In some cases, one chamber is used (instead of using separated compartments) because membrane causes a pH gradient in the reactor between the electrodes. In addition, membrane leads to ohmic resistance and consequently increasing energy consumption [68]. Concerning membrane usage in MES, the advantage of membrane-less single chamber reactor includes cutting down the cost, reducing potential losses caused by the membrane, and enhancing energy recovery [78]. On the other hand, several selections can be considered concerning membrane usage. Generally, controlling operating conditions at cathode chamber, such as pH and concentration, without influencing the anode electrolyte and avoiding mixing of the fluids are the advantages of membrane chambers, but it increases the overpotential of the system.

The membrane itself causes overpotential in the system. Around -400 to -1000 mV is required to compensate energy losses as a result of electrodes and ion exchange membranes overpotentials [79]. Although H-type bioreactors are common in MEC, the tubular chamber can also be used alternatively. In such a configuration, two concentric tubulars are used, the inner tube (contained anode) can be radially perforated (the cation exchange membrane is covered around it) and placed into the outer one. The cathode can be inserted around the membrane [80]. Restriction of oxygen diffusion is one of the main reason for using separated chambers. Oxygen produced at the anode is able to consume electrons, leading to reactor efficiency reduction. Additionally, some

bacteria are sensitive to oxygen. In a system with gas flushing, in order to lessen the reaching oxygen to the cathode, the anode can be located at the top of the reactor, so the gas flushing can diminish the oxygen flux in membrane-less reactor configuration [81]. A number of materials as membrane have been suggested for MES, commonly polymer electrolyte membranes (PEMs). In this category, Nafion as a proton exchange membrane is the reference, however, its high cost is the drawback. Ultrafiltration membrane can be an alternative which is less expensive and has high ionic resistance [59]. The other alternatives are Hyflons, Zirfons, and Ultrexs CMI 7000 as proton exchange membranes. Sulfonated polymer membranes, such as sulfonated polyether ether ketone (SPEEK), and disulfonated poly (arylene ether sulfone) (BPSH) membranes, and nano-composite polymer membranes could be used in MEC to compensate the high cost of Nafion as they are being used in microbial fuel cell [82]. Although it seems that in such a system, the use of a membrane is unavoidable, the costs of the membrane are challenging, and finding alternatives can be a future research field.

2.5 Mechanistic Study

A complex metabolic pathway in *C. acetobutylicum* from glucose to butanol and ethanol has been explained by Lee et al [41]. Metabolism of ABE-solvent (Acetone-butanol-ethanol) fermentation using *Clostridia* is performed by two different pathways: acidogenesis (acid-production like lactate-acetate-butyrate-propionate) and solventogenesis (solvent-production). Two routes were proposed by Jang et al. [83] for the synthesis of butanol: cold channel (butanol formation by direct route) and hot channel (acid reassimilation route). Electron uptake by bacteria affects solventogenesis and acidogenesis pathways [54].

Kim et al. [84] showed that applying electrochemical energy to reduce equivalent in *C. acetobutylicum* culture causes increased butanol and decreased acetone production compared to the microbial control system. Metabolic activities of microorganisms are carried out by utilizing a substrate. At the anode, this metabolism leads to the generation of reducing equivalents (protons (H^+) and electrons (e^-)). The positive ions and electrons move to terminal electron acceptors and reduced chemicals will be produced at the cathode by biocatalytic reactions. In the case of abiotic generating equivalents, strains only metabolize at the cathode. In the case of microbial electrolysis, an increase of bio-butanol production by assisting electricity has been demonstrated by *Clostridium beijerinckii* IB4 from glucose in MES. Electrofermentation leads to enhancement of butanol productivity, yield, concentration, and proportion from solventogenic species by adding electron carriers. Although total NADH and NAD^+ do not affect metabolism, higher NADH/NAD^+ ratio leads to higher butanol production [54]. *Clostridium pasteurianum* (as an electroactive heterotroph) accepts electron directly (without exogenous mediators) from the cathode which results in more reduced chemicals from organic materials. In fact, by reducing the NAD^+/NADH ratio, the intracellular reducing power increases [56]. NAD^+ and NADH are co-factor for biological metabolism. This redox couple influences the production of targeted compounds in anaerobic metabolism [57]. NADH as reducing power agent can be produced by glucolysis and utilized to produce final components [54].

In cathode compartment, the following reactions take place on biofilm (biocathode) (adopted from Logan et al. [19]):

Equation 2-6 $\text{Organic matter (Glucose)} + ne^- + nH^+ \rightarrow \text{alcohols/solvents such as butanol}$

Equation 2-7 $4CO_2 + 24H^+ + 24\,e^- \rightarrow butanol + 7H_2O$ [29]

A reduction mechanism of glycerol was explained by Zhou et al. [31] as follows:

Equation 2-8 $Glycerol + NADH \rightarrow 1,3\ PDO + H_2O$

Non-reductive metabolic comprises conversion of glycerol to succunic acid, lactic acid, water, 2,3-butanediol, carbon monoxide, acetic and formic acid, hydrogen and butyric acid.

In addition, at the cathode, carbon dioxide as a carbon source can be transformed into acetate and water by consuming hydrogen ion and electron.

In anode chamber, the reaction can be abiotic (not depending on the substrate) or biotic, the following reactions occur:

Equation 2-9 Organic matter + H_2O (may involve or not) → electron+cation+nCO_2 + (acetate/ethanol)

Equation 2-10 $2H_2O \rightarrow 4e^- + 4H^+ + O_2$

Polymeric materials also can be converted to organic acids or alcohols by hydrolysis and fermentation in anode chamber.

MES relies on extracellular electron transfer (EET) [17]. EET is the process in which bacteria transfer electrons into and out of the cell from or headed for electron donor/acceptor. At anode compartment, the first assumption (which is a common idea) is electron transport from organic matter as electron donor towards minerals and electrodes. The direct electron transfer from strain to the anode (as a consequence of oxidation of substrate) is clear but still, the reverse transportation mechanism from cathode microorganisms has not been fully understood [65]. Direct cathodic EET (direct biocatalysis) which includes direct contact between microbes and cathode and electron transfer through nanowires and electron transportation through hydrogen are known for electron transfer mechanism from the cathode to microorganisms. The other option of cathodic

EET is via electron shuttles (endogenous mediator shuttled and artificial mediator shuttled), and the fourth one is indirect electron transfer via intermediate building blocks (by-product shuttled), such as formate or via extracellular polymeric substances (EPS) [10, 42].

As mentioned earlier, nicotinamide adenine dinucleotide as a coenzyme (reduced form NADH) plays an important role in MES. Reducing equivalents affect the biochemical yield and cellular growth rate. Moreover, balancing the redox intermediates (such as NADH) is a crucial concern in metabolic engineering. For this purpose, the bioelectrochemical approach is a technique to improve redox balances in the systems of bio-solvents and biofuels productions. Reducing intracellular NAD^+ to NADH can be performed in such a system to balance the cells' redox. This bioelectrochemical method can be used to enhance yields and/or productivity of final products in the fermentation system. For instance, using *E. coli*, and by utilizing substrates such as glucose, xylose, glycerol, maltose, sorbitol, and gluconate, the number of NADH produced are 2, 1.67, 2, 4, 3, and 1, respectively. On the other hand, The number of NADH consumed by different products such as succinate, 1,3-propanediol, 1,4-butanediol, n-butanol, and ethanol are 2, 3, 6, 4, and 2, respectively which can be improved by BES [85].

Electron addition through the cathode into a microorganism results in enhanced reduction reaction by increasing a reduced cofactor such as NADH as the following equation:

Equation 2-11 $$NAD^+ + H^+ + 2e^- \rightarrow NADH$$

For instance, *C. pasteurianum* could accept the electron from the cathode and consequently enhanced notably the production of net NADH-consuming products such as butanol (by glucose fermentation) and 1,3-propanediol (1,3-PD) by glycerol fermentation. In fact, total NADH consumption for butanol production showed that metabolic pathway shifted to butanol reduction

pathway by electron supplement in MEC, and the results indicated that total NADH consumption was higher than control fermentation. Electron and NADH balances disclosed that electrofermentation for production of biofuels and chemicals can open the great door to the future [39,42, 56].

NADH consumption can be calculated by computing the number of NADH needed for each metabolite per mole: NADH consumption = $n\ \Delta P$, and NADH generation is computed by 2 ΔS (mmole) from the substrate. In addition, NAD^+ is measured by using an enzychrome NAD^+/NADH Assay Kit [56]. Choi et al. [56] claimed that lactate was produced in MEC only (synthesis of butanol from glucose by *C. pasteurianum*) compared to a non-electrical system which shows the microorganism accepts an electron from the cathode by changing the fermentation behavior. It can be concluded that butanol formation increases by consuming lactate. The other factor which influences the MES mechanism (biologically point of view) is ATP. The source of Adenosine Triphosphate (ATP) which affects the cell growth in ABE fermentation is done via acetic and butyric acid production. He et al [54] reported that in MEC, the ATP level was a little lower than the control system, but by adding neutral red (NR), its value increased due to the transport of electrons onto the cell.

Understanding the impact of electron transfer routes and mechanism of energy conservation is significant to study MES. The degree of reduction (DoR) (for direct conversion) demonstrates the electron demand of the product. It can be calculated by the following formula for generalized compound $C_aH_bO_cN_dS_eP_f$:

Equation 2-12

$$\mathrm{DoR} = \frac{4a+1b-2c-3d+6d+5f}{a}$$

where C: +4, H: +1, O: -2, N:-3, S: +6, P: +5. Glucose with DoR=4 by electron supply can be converted to higher DoR compounds like alcohols [86]. In MES, in the anode chamber, the electron goes from microbes to electrode and in the cathode chamber, the direction of electron transport is reverse. In fact, the electrons transferred from electron donors to electron acceptors (from lower redox potential to higher redox potential) [42]. The stoichiometric approach is necessary to determine the redox balance in the MEC system. In some cases, there was no increase in yield with increasing redox equivalent, though overall limited predictive power of the DoR of products had a higher degree of reduction compared to the substrate (like ethanol). Besides, it was observed that when the reductive state was equal, substrate-product-combinations got to benefit from extracellular electron supply (like3-hydroxy-propionic acid). Several biochemicals and alcohols display increased production by extracellular electron supply at the cathode. For instance, propanediol production through MES depends on EET mechanisms. 1,3-PDO production from glucose and glycerol showed that glucose needs the double amount of reducing equivalents than glycerol. The electron can transfer from the cathode to the microbial cell by two different mechanisms. By the mechanism of oxidation on outer membrane cytochromes and simultaneous ATP generation (which leads to transferring the electrons into the organism and finally onto NAD), the substantial improvement of the 1,3-PDO yield (up to 92.9% by using glucose) can be achieved. However, if the electrons and protons are transported directly onto NAD (without ATP synthesis, by diffusion into the cytoplasm or catalyzed by enzymes such as hydrogenases), the yield of the product could increase up to 62.5% due to energy limitation. For 1,3-PDO production from glycerol, both mechanisms lead to the yield of 100% maximum [86].

Fig. 2.1 shows the overall view of solvent production by glucose and glycerol substrate by clostridia species which is catalyzed by electron receiving from the cathode. Synthesis of organic carbon like glucose to pyruvate (CH_3COCOO^-), the anion of pyruvic acid is the first step of

metabolism which is followed by the formation of acetyl coenzyme A ($C_{23}H_{38}N_7O_{17}P_3S$), and during the following steps, ethanol (C_2H_5OH) and acetone $(CH_3)_2CO$ will be produced and butanol (C_4H_9OH) is formed at the end of the synthesis pathway.

Consequently, reduction of NAD^+ to NADH and $NADP^+$ to NADPH leads to higher solvent production in MEC by use of mediators or without using mediators depending on electron transfer mechanism from the cathode to microorganism.

2.5.1 Electron mediators

Redox mediators are chemicals which are able to accept an electron from the cathode and deliver to bacteria by reduction and oxidation. Mediators (electron carriers), such as methyl viologen (MV), benzyl viologen (BV), methylene blue (MB) and neutral red (NR) are able to transport electrons to biological compound and change the bio-reaction pathways [54, 57]. For instance, NR leads to the metabolic pathway in ABE fermentation to ethanol and butanol [87]. Butanol production also increased by adding NR in a study by He et al. [54]. Four electron carriers were examined in the presence of *C. beijernkii* IB4, among them, only NR showed the best result for butanol production and lower acetone production. Actually, NR can be reduced in the acidogenesis phase, whereas it can be oxidized in the solventogenesis phase. Choi et al [57] showed that butyrate production (rather than hydrogen and acetate) enhances by the aid of MV in the electromicrobial synthesis using *C. tyrobutyricum* BAS 7 via changing electron flow, while NR and AQDS had no significant effects. In a study conducted by Steinbusch et al. [58], MV and NR enhanced the production of ethanol, but only MV was a successful mediator for increased concentration of ethanol in the product. By affecting NADH in microbial fermentation, the power to producing chemicals can be changed. Intercellular NADH and NAD^+ concentrations can be

measured by enzyme cycling procedure. For butanol formation, NADH is a constraint. If four NADH oxidize to NAD^+, one mole of butanol is produced. Electrically NR reduces NAD^+ to NADH, thus it leads to an increase in the ratio of NADH/NAD^+ during the fermentation process. As another result, hydrogen produced in clostridia metabolism can be catalyzed by hydrogenases that competes with the main product, but by adding NR, H_2 production decreases [54]. In a study, by adding electron carriers, acetone production decreased, which resulted in improved the B: A ratio. Moreover, the maximum butanol production (18.20 g/L and 18.05 g/L) achieved by a combination of methyl red and butyric acid, and benzyl viologen, respectively. Also, benzyl viologen and butyric acid addition led to a B: A ratio of 16:1 which means 8 times intensification compared to traditional ratio [88].

Therefore, understanding the electron transfer mechanism of microorganism in MEC is a key to choose electron mediators to increase specific product formation.

2.6 Conventional biological process vs MES

Fig. 2.3 shows the production of different chemicals using MES in comparison with conventional fermentation method. 1,3-propanediol can be produced by glycerol fermentation using *Clostridium* microorganisms. Integration of current and metabolism of microbes leads to improvement in yield and redirection from propionate fermentation to 1,3-propanediol production. This way, the production rate of 1,3-propanediol enhances during bioelectrosynthesis (50.1%) compared to the process without using current (24.8%). In addition, with hydrogen supply 1,3-propanediol production increases [31]. Xafenias et al. [55] reported that the production rate of 1,3-propanediol increased up to 6 times and a higher concentration of product was achieved by the assistance of bioelectrosynthesis in comparison with controlled microbial synthesis. Actually, the glycerol electrofermentation is supported by a biofilm of microbes and bacteria on the cathode, but

in the non-electrochemical process, the performance was dramatically low. Ethanol production rate, efficiency and concentration were enhanced by applying MES in the presence of MV due to inhibition of side processes, such as methanogenesis and n-butyrate production in a mixed culture [58]. Choi et al. [56] investigated electro-microbial fermentation of glucose and glycerol by gram-positive *C. pasteurianum*. They showed that by bioelectrolysis of glucose, butanol production enhanced (13.5 vs 5.4 mM) and acids production, such as acetic and butyric acid decreased. Also, optical density (O.D) and growth rate decreased due to the formation of biofilm on the cathode. This way, the metabolism shifted to the reduction pathways using MES. When glycerol was used as the substrate, the result demonstrated increased PD production as a major product rather than butanol. In fact, more current consumption changed metabolic pathway to reduce glycerol to NADH-consuming compound. In recent research by He et al. [54], fermentation of glucose to butanol was carried out using *C. beijerinckii* with the assistance of electricity and electron carrier. For solventogenic phase, the utilization rate of glucose increased from 1.43 to 1.81 g/L/h at control (conventional fermentation) at poised electricity of -700 mV (vs Ag/Agcl reference electrode) respectively. The values here reported as 1.67 and 1.81 in case of neutral red (NR) addition and electricity reduced NR. Productivity and yield of butanol were enhanced by the electricity-driven system and by adding an electron carrier.

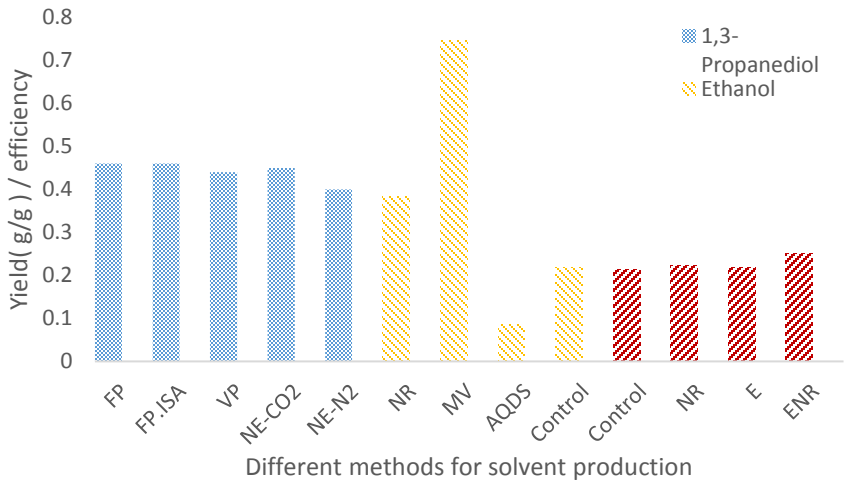


Figure 2-3 Yield (solvent g/substrate g) of 1,3-propanediol production (blue bar, left side); Ethanol efficiency (yellow bar, middle), and butanol yield (red bar, right side) in MEC [48,49,52]. Neutral red (NR); methyl viologen (MV); anthraquinone-2,6-disulfonate (AQDS); fixed electrode potential (FP); fixed-potential increased electrode surface area (FP-ISA); varying potential (VP); non-electrochemical CO_2 spared (NE-CO_2); electrochemical N_2 spared (NE-N_2); electricity of cathodic potential (E); electricity reduced NR (ENR).

Efficiency enhancement is one of the major challenges in conventional fermentation, and many researchers focused on the improvement of production rate using low-cost materials in MES. Giddings et al 2015 concluded that MES can be supplied with DC power sources at high columbic efficiencies. Using a simple membrane-less reactor, and applying the less expensive of DC power sources than the potentiostat-controlled system at the cathode (especially in industrial scale) can provide the basis of design for MEC [81]. In general, although the studies showed strengthens in yield and productivity by MES for solvents and alcoholic production compared to traditional fermentation, controlling system, capital cost, and optimization of operating parameters are among the main challenges.

2.7 Techno-economic reports

Tao et al. [89] have done a techno-economic evaluation of n-butanol production using *Clostridia* (ABE conventional fermentation) and corn as a substrate. Total capital included feed, raw materials and storage, saccharification, fermentation, recovery, separation, product storage, utilities and waste treatment, equipment, direct and indirect costs. If operating cost was taken into account, the minimum butanol selling price would be $ 2.31 to 2.98 gal considering coproduct credits (ethanol, acetone, and hydrogen) based on 2007 US dollars. Also, the average return on investment would be $0.38 /gal butanol. In addition, considering corn stover as feedstock, the final price would be $3.33 gal including by-product credits (ethanol, acetone, and electricity) and an average return on investment equals $1.44 /gal butanol. Moreover, other economic calculations for bioelectrochemical systems [90-91] showed different expenses, such as fermentation, water evaporation, transportation etc. are important factors. Sustainability assessment in a biorefinery system showed that MEC has some advantages, such as internal hydrogen production from waste, production of valuable chemicals and make-up water reduction [92]. As can be observed, the techno-economic study of MES technology has not been done at industrial scale for solvent production, thus feasibility study must be performed in future to evaluate the final product cost, energy consumption, and feasibility of the establishment of MEC industry. MES technology should answer this question that the electron supply approach is more economical (by considering the targeted product) compared to conventional fermentation.

2.8 Scale-up considerations and constraints

Nowadays, 1-butanol is mostly produced in petrochemical industries. Alternatively, biobutanol is produced through two-stage ABE fermentation at industrial scale by solventogenic *Clostridia*.

Also, it has been mentioned that commercially solvent yield is about 0.35 g/g sugar [93]. According to Escapa et al. [94], three scenarios for wastewater treatment by MES were proposed and they finally concluded that optimizing (reducing) current density and energy consumption played important role in capital investment for reactors. In the case of hydrogen production, the price of H_2 was more significant than the size of MEC and electricity usage. Moreover, geometric surface area-electrode/reactor volume-ratio and optimization of flow regime are important factors in designing MES [95].

In general, the steps for commercialization of MES technology starting from bench scale comprises step 1: determination of targeted product, substrate, microbial strains, medium, performing voltammetric study, redox test; step 2: setting up experimental system (reactor type, electrode materials etc.) and design of experiment, taking data and specifying the most important parameters such as pH, concentration, and temperature; and step 3: optimization, system control, recovery processes selection and cost evaluation of pilot, doing experiment in pilot and planning to demonstration scale.

Technical, economic, and environmental feasibility must be considered in this technology as well. Reduced capital and operational cost compared to the conventional process of wastewater treatment, and compatibility with environmental policies must be explored. In a study which has been performed for hydrogen production using wastewater (WW), it is estimated that energy value recovery is \$0.19 kg^{-1} COD and \$0.06 kg^{-1} COD for domestic WW and winery WW, respectively. Cathode material and membrane are the most important factors for techno-economic calculation of MES. To reduce the capital cost, the platinum electrode can be substituted by less expensive materials, such as stainless steel, nickel or MoS_2-based [13]. To industrialize the aforementioned system, focusing on a specific high efficacy and engineered microorganism strain is required for

the specific product, such as butanol as applicable alcohol, solvent, and fuel. Small or well-known companies are interested in the capitalization of MESs, therefore cost-effective materials and designing criteria are important for commercialization of this system [68]. As reported by Logan [68], the pilot scale of MEC was tested for hydrogen production by use of winery wastewater in the USA. Moreover, the pilot scale of microbial electrolysis cell with 100 L and 120 L volume for producing hydrogen was tested using domestic wastewater for about one year and 3 months, respectively [96-97]. In a proposed process by Lovley and Nevin [29], CO_2 and H_2O as feedstock entered the microbial electrolysis reactor and O_2 was released. The liquid effluent from the reactor goes to the continuous gas stripper with carbon dioxide as another input stream, the stripper equipped with a condenser which included gas recycle and a product outlet.

Traditional fermentation technology at the commercial level has several constraints such as, relying on pure substrates, specific culture medium specific (in most cases) for each microorganism, redox-imbalanced which confines the product selectivity, and pH control that often limit the application, environmental sustainability, and economic expediency.

Advantages of MEC goes to innovative industrial fermentation processes and might be a suitable way of waste biomass utilization towards added-value biorefineries. Using MES, the fermentation process is able to be controlled and optimized to achieve products with higher purity, to appropriate microbial cell growth and density. MES could extend the possible range of fermentation substrates such as waste organic matter, optimizing conventional industrial fermentation operation, decreasing chemical addition for controlling the process, for example, pH, redox regulation, antifoaming agents, etc., and broadening the range of high-value products [98].

High capital costs of MES technology are the main obstruction to scale-up and industrialization. It is estimated that a MEC (by using the materials in the laboratory, such as platinum-based cathode

and Nafion membrane) leads to capital costs maximum 800 times higher than anaerobic digestion. In fact, membrane and cathode involve up to 85% of the capital cost (cathode: €500 m^{-2}, and membrane: €400 m^{-2}). Ultrex membranes (€110 m^{-2}) and Zirfon (€45 m^{-2}) type of ion-permeable membrane are alternatives for Nafion membrane. Moreover, non-ion-selective membranes can be used in single-chamber bioreactors to avoid electrical contact between the electrodes [13]. Thus, decreasing cost of materials (such as membrane and electrodes) by use of alternatives (e.g. carbon electrodes instead of platinum ones) seems unavoidable. Moreover, to commercialize the MEC process, several parameters should be considered, such as product yield, final concentration, the rate of the product, and efficiency in terms of energy. Product yield which represents the efficiency of a system is one of the important parameters. The final product concentration or titer is also another crucial factor that determines the purification cost. The rate relating to system volume also affects capital cost. The energy efficiency (the amount of energy consumed per kg of product) needs to be attended to reduce the costs. Last but not least, operating costs, such as electricity and power cost, substrate price, and separation cost should be considered [8]. In a study, it was estimated that biobutanol production from corn and by using *C. beijerinckii* BA101 costs $0.34/kg. An enhancement of biobutanol yield by 19% (0.42–0.50 g biobutanol per g glucose) results in a reduction in the final biobutanol price to $0.29/kg [53].

2.9 Current and future development

Biofuels can classify in three groups based on their production technologies: First generation biofuels such as bioethanol (from sugar, starch, vegetable oils, or animal fats); second-generation biofuels (from nonfood crops, wheat straw, corn, wood, solid waste); third generation biofuels (from algae); and fourth generation biofuels (conversion of veg-oil and biodiesel into biogasoline) [33]. As

mentioned earlier, biofuels and biochemicals can be made from CO_2 or organic biomass. Each source has its pros and cons. Using CO_2 benefits from excess availability, and removing CO_2 from the atmosphere has a positive impact on the greenhouse effect. Also, uptaking CO_2 by the cell does not need an investment of energy, and it is not dependent on arable lands. However, the formation of products requires a high number of electrons. When organics are used, the limited electron is required since substrates are partially reduced, also it has a beneficial impact on the environment when using wastes. Nonetheless, energy is needed for transport or activation, it has an unfavorable pH effect in the system depending on the source, it requires arable lands, and its availability depends on the location [23].

Different process integrations for production of value-added chemicals are possible, such as separate hydrolysis and fermentation, simultaneous saccharification and fermentation, simultaneous saccharification and co-culture fermentation, and consolidated bioprocessing or direct microbial conversion. The simultaneous saccharification and co-culture fermentation technique is the most developed method as hydrolysis and fermentation are done in one stage. Fermentation suffers from several hindrances, like inhibition effect of by-products and products (acetates, furfural, ethanol etc.), and low conversion rates. To overcome, ethanologenes such as yeasts cab be utilized to convert C_5 sugars, and also some microorganism, such as *E. coli* can be used for mixed sugar fermentation to produce bioethanol. Regarding biobutanol production, several industrial stakeholders have investigated novel alternatives to commercialize this process (rather than conventional ABE fermentation). *The two leading technology developers in this area are Gevo (*developing *E. Coli* strains for converting keto acids into aldehydes, and aldehydes into 1-butanol*),* and Abengoa method (catalytic condensation of ethanol to produce butanol) [99].

Scaling up the MES is more feasible than MFC because the high value-added products can be produced in this way. Likewise, it can address fossil fuel alternative as the main advantage, but there are challenges which should be solved [100]. For instance, a low rate of electron consumption by bacteria at the cathode is one of the molecular challenges [9]. To optimize the energy generation and productions, synergistic interaction between strains and components should be studied and well understood [101].

Butanol yield by conventional anaerobic fermentation is not optimal in spite of existing large scale processes [102]. As 1 kg butanol production requires approximately 3 kg of glucose [23], using brewery wastewater would be a choice for producing alcohol by MES. There are economic constraints for electricity-driven bioproduction due to reactor construction, biological challenge, and electricity needed [23] which could be considered for pilot and plant design. Rozendal et al [17] estimated that capital cost of MES in laboratory scale comprises electrodes and membrane, but in future, it contains current collectors, membrane, and reactor. One challenge in MES is low voltage efficiency which can be improved by decreasing overpotential and applied a voltage to increase current densities [70]. In addition, the cost of materials, efficient reactor configuration, and reaction rates are still the challenges for scaling up the MES [103]. In an experiment in pilot scale (1000 liter), microbial electrolysis cell was tested in continuous flow fed winery wastewater to produce electricity and removing COD. It showed that inoculation, pH and temperature were the critical parameters for improvement of the reactor performance [104]. The solution to the current complications facing MES technology can be speeded up by integration of microbiology and electrochemistry along with the suitable mathematical model and presenting an efficient control system [6]. A number of strategies have been carried out to improve butanol production as one of the main products of solventogenic MES, such as using mixed substrates, hyper-butanol strain screening, continuous process, organic acid addition and in-situ butanol recovery [54]. Also, if the

production of butanol increases from 12 to 19 g/L by improving butanol tolerance, the separation expense will dramatically decrease from the economical viewpoint [35]. Thus, real mixtures such as wastewater from sugar/biodiesel/winery/brewery industries in laboratory scale and pilot plant scale can be explored. In addition, economic evaluation should also be done as a part of the feasibility study of large scale MES plant. Furthermore, using another bacteria which are able to produce electron from the substrate (such as *Enterobacter*) can also be an alternative to enhance the efficacy in anode chamber while reduction takes place at the cathode to produce solvents. Electro-fermentation (EF) is able to optimize microbial processes and has an impressive effect on emerging biomass refinery chains. More details about the electrical potential and current impact on the organism metabolism and microbial strains related to EET must be more understood [98].

2.10 Electrochemical conversion of glycerol into value-added products

Nowadays fuels and chemicals production from bioresources has come to be a high significance because of the problems related to fossil fuels, like dependence on external resources, and adverse environmental influences. In fact, biofuels produce the inferior amount of detrimental gas emissions such as carbon monoxide and NO_x, and lower level of greenhouse gas formation during production and usage compared to petroleum-derived diesel due to their originality from biomass. Biodiesel is the most frequently used biofuel since it can be utilized in normal diesel engines without adjustment with low-cost alterations. Historically, biodiesel consumption for transportation services has been growing quickly since 2004 with an average annual growth rate of 70%, (for instance, increasing from 91 million gallons in 2005 to 412 million gallons in 2008).

Classically biodiesel is produced by transesterification process (by acid or base as a catalyst) by using animal fat or vegetable oil as feedstocks. Such a process leads to 10% crude glycerol

production. As biodiesel usage is increasing, the production of glycerol is anticipated to rise meaningfully. Thus, this cheap byproduct can be used to produce value-added chemicals. One of the most favorable routes for glycerol conversion into valuable chemicals is liquid phase oxidation. For thermochemical methods, controlling the selectivity and enhancing efficiency need altering temperatures and pressures, which leads to increasing the operating costs, sharply. Otherwise, electrochemical procedures have the potential to use electricity from renewable resources and can be operated at moderately low temperatures and pressures, which results in removing heat requirement or pressuring vessels of the process. Furthermore, selectivities can be controlled by regulatory the applied potentials, pH of electrolytes, and selection of electrocatalysts [111].

The impacts of electrode materials, such as gold, platinum, palladium, and nickel, have been studied in alkaline media to produce a varied range of chemicals, including glycerate, oxalate, glycolate, tartronate, carbamate, and formate, etc. and the deficiency of product selectivity is observed. Also, an extra basic solution may also lead to corrosion of the electrochemical reactor during the actual operation. According to literature, there is a lack of information concerning the electrochemical conversion of glycerol in an acidic electrolyte. While there have been reported electro-conversion of glycerol in acidic media, the research only focused on the electrochemical behavior study based on the effects of the electrode material, electrode potential, and voltammetry studies; electrochemical investigation in galvanostatic mode in a large volume of bench scale has been seldom debated in the literature [112]. Table 2.2 shows the recent study in this field.

In a recent study, it has been demonstrated selective glycerol-to-1,3-PD conversion using Pt or RuO_2-based as anode and Zn or Pb as a cathode in NaCl and KCl at pH 1 [113]. In another study, it was shown that an electrochemical dehydrogenation process can be used to oxidize glycerol to glyceraldehyde and glyceric acid even without using stoichiometric chemical oxidants in an electrocatalytic batch reactor [114]. In an investigation, it was reported that a carbon supported

platinum electrode in a bismuth saturated solution is capable of oxidizing glycerol to dihydroxyacetone. In the absence of bismuth, the primary alcohol oxidation is dominant but Bi blocks the pathway for primary oxidation [115]. A study by Au nanoparticles supported on extended poly(4-vinylpyridine) functionalized graphene (Au-P4P/G) in alkaline media showed a much higher activity and much better selectivity for three carbon products and the results indicated that Au nanoparticles induce higher three carbon selectivity and the changed adsorption ability for oxygen-containing groups might be the main reason which resulted in different organic acid production like glyceric acid, glycolic acid, tartronic acid, oxalic acid, formic acid [116].

Table 2.2. Various recent works on electro-oxidation/reduction of glycerol

Feed and process	Electrodes and reactor type	Operating conditions	Products and conversion	Ref.
Electrochemical conversion of Enriched crude glycerol	Galvanostatic mode, Pt electrode	pH=1 Current density= 0.14 A/cm2 Ambient T and P t=14 h	ethylene glycol, acetol, glycidol, acrolein, 1,2-propanediol (PD) and 1,3-PD complete conversion of 0.3M glycerol within 14 h with a total product yield of 68.7%	[117]
Selective oxidation of glycerol	Electrocatalytic dehydrogenation working electrode was a catalyst-coated carbon paper (active area: 4 cm2) with 20 wt% Pt/C (1 mg Pt-metal).	t=10 h T=60 C 0.1M glycerol in 0.5M H_2SO_4 and from controlling electrical potential Anode Applied Potential [V vs. SHE]= 1.097 Glycerol/Pt molar ratio=195	glyceraldehyde and glyceric acid (87% selectivity) 91.8 % glycerol conversion	[114]
Selective Electro-Oxidation of Glycerol	carbon supported platinum electrode in the Presence of Bismuth	0.5−0.6 V vs NHE Glycerol 0.1 M Acidic solution 0.5 M H_2SO_4	Dihydroxyacetone high selectivity glyceraldehyde, and glyceric acid. By-products	[115]
Electrochemical reforming of glycerol solution	Pt electrode	Galvanostatic condition (A=4.5 A), pH=1 ambient T and P	propanediol, glycidol, and 2-propenol etc.	[118]

Glycerol oxidation	nickel-based nanocatalysts	Alkaline medium (0.1 M NaOH) chronoamperometry condition. two set potentials, 1.6 V and 1.9 V vs. RHE, during a period of 8 h.	Glycerate, glycolate and formate	[119]
Electrocatalytic Glycerol Oxidation	20 wt % Pt/C	0.1-1.0 M glycerol in 0.5 M H2SO4 0.9 and 1.1 V (vs SHE) Glycerol conversion= 15.6-89.5 % during 10 h operation T=60 oC	GAD, glyceraldehyde; GLA, glyceric acid; HPA, hydroxypyruvic acid; TTA, tartronic acid; GCA, glycolic acid; OXA, oxalic acid. And hydrogen	[120]
selective transformation of glycerol to dihydroxyacetone	PtSb/C catalysts	0.1 M glycerol; anode applied potential: 0.397 V to 1.197 V, At 0.797 V; temperature: 60 °C, glycerol conversion of 90.3%	dihydroxyacetone (DHA), glyceraldehyde (GAD), glyceric acid (GLA), and glycolic acid (GCA).	[121]
Electroconversion of glycerol in alkaline medium	bimetallic M50@Pt50 nanocatalysts (where M ¼ Ru, Sn or Ni) supported on multi-walled carbon nanotubes	Onset oxidation potential (275 mA. Pt and _492mV vs. Hg/HgO/OH) 12 h electrolysis 40-60% glycerol conversion	Formate, glycerate, glyoxalate, tartronate, oxalate	[122]
Selective electro-oxidation of glycerol	anion-exchange membrane electro-catalytic flow reactor Au nanoparticle catalyst	anode potential from 0.35 to 0.9 V vs SHE, 50◦C glycerol concentration was reduced from 1.0 to 0.1 M t=max 18 h	Tartronate Mesoxalate Glycerate Oxalate	[123]
Selective electro-oxidation of glycerol	Au nanoparticles supported on extended poly(4-vinyl pyridine) functionalized graphene	0.2-1.9 V (vs. HgO/Hg room temperature 0.5M NaOH+0.5M glycerol Solution Conversion 50%	glyceric acid glycolic acid tartronic acid oxalic acid formic acid	[124]
glycerol electrooxidation	Ni-based materials	Room temperature (21 °C) 0.1molL⁻1glycerol +0.1molL^{-1}NaOH 1.6 V vs.RHE Glycerol Conversion 12.9-17.9%	Formate Glycolate Glycerate	[125]
selective electrochemical conversion of glycerol	RuO2-dsa and Pt anode; Pb and Zn cathode. Divided and undivided batch cells	In Kcl, NaCl, and HCl 0.5 M base solution and 0.25 M glycerol Potential -1.8 V or 2.5 V vs. AgCl/Cl sat. KCl, temperature 25 _C).	1,3 PD Acetol acetone	[113]

2.11 Conclusion

Biomass nutrients in wastewater are a renewable and alternative source for the production of fuels and chemicals. Nutrient waste, such as crude glycerol (a by-product of biodiesel production) could be known as a source of energy which can play a major role in the future of world energy. This review provided a survey of producing chemicals especially alcohols by integrated microbial fermentation and electrochemical synthesis. An interaction mechanism between electroactive microorganisms and electrodes via extracellular electron transfer and intercellular metabolism in the presence of excess electrons in the electro-bioreactor are also reviewed. Since there are various drawbacks of microbial fermentation of alcohols, solvents, or biofuels, such as low product concentration and productivity and the high cost of recovery, the electron-assisted process would be an alternative to enhanced yield. Fermentation of butanol as one of the useful and important products of solvent fermentation should be viewed from an economic point. To carry out the same, using inexpensive organic materials, improving fermentation methods (e.g. MES) and efficient product recovery are known. Using mediators is another alternative to enhance yield and productivity along with electricity in the MES system. Co-culture techniques (growing of two or more cell types in a culture which a microorganism acts as a reducing agent for the other bacterium that leads to improved production efficiency), genetic modification of bacteria (*Clostridia* species which can tolerate high butanol concentration), and in-situ separation of products are other options for improving the solvent fermentation that can be combined with MES to obtaining higher yield and achieving economic feasibility. Techno-economic evaluation is also required for MES as it has been carried out for conventional fermentation of biohydrogen and biochemicals. Chemical and fuel synthesis from the waste in MECs can be emerged as a solution to environmental issues and energy problems, though it has not been commercialized yet. The review indicated that the economical electrode materials with the capability of efficient electron transfer could be carbon-

based electrodes which are beneficial due to their good stability and low cost. Also, it can be concluded that special requirements of MES reactor system are an autoclavable reactor, electrodes, and separators (membranes), which should not be neglected in bioreactor design in terms of efficiency, and economic aspects. As MES for alcoholic biofuels is in infancy (major studies were performed on hydrogen production), the production of solvents and alcohols in MEC through synthetic media and real waste is currently underway. To sum up, by MES some constraints can be removed in comparison to traditional fermentation and goes towards more selective products.

Glycerol is a highly versatile molecule due to its three hydroxyl groups and it is able to be converted to different value-added chemicals. It is a byproduct of the biodiesel industry, so, produced in high amounts, which led to a high amount of production in the market over the last decades. Glycerol has the potential to be considered as a platform chemical. The selective oxidation/reduction reaction is one of the most promising reaction routes to produce valuable fine chemicals used in the chemical and pharmaceutical industry. Moreover, during the past decade, scarcely electrocatalytic pathways for glycerol oxidation have been studied, therefore studies on the different aspect of the process such as applied current density, electrode material, and pH must be more studied to optimize the process.

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CHAPITRE 3

Enhancement of biobutanol production by electromicrobial glucose conversion in a dual chamber fermentation cell using *C. pasteurianum*

Amélioration de la production de biobutanol par la conversion électromicrobienne dans une cellule de fermentation à double chambre avec *C. pasteurianum*

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3 Enhancement of biobutanol production by electromicrobial glucose conversion in a dual chamber fermentation cell using *C. pasteurianum*

3.1 Abstract

A set of experiments have been performed to investigate the production of biobutanol as a novel applicable biofuel in a bioelectrolysis cell (BEC). The objective of this work is to understand the mechanism and production rate of the biobutanol by bioelectrosynthesis (BES) using glucose as a substrate. Four main factors, such as electrode materials, substrate concentration, operating temperature, and poised applied voltage were investigated in batch mode to achieve optimum condition for producing maximum butanol by *C. pasteurianum* in BEC. Standard modified P2 medium (MP2) and standard minimal medium (SMM) were used as fermentation media in batch operation mode. Numerical optimization using a central composite design (CCD) method has been used to maximize the butanol production within the experimental range. The maximum butanol production 13.31 g/L was obtained by applying 1.32 V indicating the suitability of this procedure. The results showed that by applying optimum conditions in SMM, the butanol could be enhanced remarkably by electroactive microorganisms in the cathode chamber.

Keywords: Bioelectrosynthesis: biobutanol: biofuel: electrode: electron transfer: optimization.

Résumé

Un ensemble d'expériences ont été réalisées pour étudier la production de biobutanol en tant que nouveau biocarburant. L'objectif de ce travail était de comprendre le mécanisme et le taux de production du biobutanol par bioélectrosynthèse (BES) en utilisant le glucose comme substrat. Quatre facteurs principaux, à savoir le matériau des électrodes (la surface de l'électrode sur le volume du réacteur, S/V = 0.083 cm^2/mL), la concentration du substrat, la température de fonctionnement et la tension appliquée ont été étudiés en mode discontinu pour fixer les conditions optimales de production maximale de butanol par *C. pasteurianum*. Le milieu standard P2 modifié (MP2) et le milieu standard minimal (MMS) ont été utilisés comme milieu de fermentation en mode de fonctionnement par lots. L'optimisation numérique à l'aide de la méthode de conception composite centrale (CCD) a été utilisée pour maximiser la production de butanol dans la gamme expérimentale. La production maximale de butanol à 13,3 g/L (contre contrôl 10.2 g/L) a été obtenue en appliquant 1,32 V, ce qui prouve la pertinence de cette procédure. Aussi, les résultats ont montré que l'opération dans les conditions optimales de MMS, la production du butanol pouvait être améliorée de façon remarquable par des microorganismes électroactifs dans la chambre cathodique.

Highlights

- Biobutanol enhancement by microbial electrosynthesis (MES).
- A new generation of biofuel.
- Optimization techniques using central composite design.
- Mechanism understanding using NAD^+ and NADH metabolic pathway and electron equivalent balance.

3.2 Introduction

The rapidly growing demand for biofuel is gaining attention nowadays, especially for biobutanol beyond bioethanol. Biobased butanol fuel is known as a second generation alcoholic biofuel which includes higher energy density and lower volatility compared to ethanol. A number of companies are looking for developing biobutanol on an industrial scale. This biofuel is able to compete with $80 bbl oil [1]. The advantage of biobutanol is its variety of commercial usages in a current market worth over $5 billion dollars [1]. Biobutanol which has such physico-chemical properties that can be properly used in compression ignition (CI) engine. Using n-butanol as pure or blends with diesel has demonstrated the promising potential for enabling operation in diesel engines. Among different fuel properties, the long ignition delay, and high oxygen content of n-butanol are key factors to amend the fuel-air mixing and lower the NO_x and ash emission [2]. The impact of biobutanol and biobutanol–diesel blends on the combustion and emission features in an ignition engine showed that the indicated specific fuel consumption (ISFC) of the mixed fuels was higher than that of diesel. Nevertheless, the exhaust temperature was less than that of diesel. Moreover, nitrogen oxide (NO_x), carbon monoxide (CO) and soot from Bu20 (20% biobutanol and 80% diesel) were lower than those values from diesel fuel, however, hydrocarbons (HC) were higher than that from diesel [3]. Biobutanol is also an important chemical and solvent which has been recently addressed as one of the evolving second generation liquid biofuel. As a liquid transportation fuel, butanol is preferred to the first-generation biofuel, bioethanol, because of its higher mixing rate with gasoline without engine modification, octane enhancing number, and suitable delivery using current pipeline infrastructure. Furthermore, its energy content is higher, since butanol contains 96% of the energy of a gasoline volume unit, while ethanol only produces 73% of gasoline energy per unit volume [4]. Apart from biofuel application of butanol, it is a global chemical with over 4 million tons of annual demand, n-butanol is an applicable chemical utilized in grave high-value

applications in a broad range of commercial markets. It is also an intermediate used to produce resins and specialty solvents used in final product formation, such as paints and coatings to cosmetics and perfumes, adhesives and inks, and even food flavours and extracts. It can be used in plastics and polymers, brake fluids, lubricants, synthetic rubber, fire retardants, etc as well. [5].

Conventional biobutanol fermentation suffers several limiting constraints. One of the main challenges of traditional biobutanol production is low productivity which can be enhanced by one of the novel techniques, such as electromicrobial synthesis (EMS) or bioelectrosynthesis (BES). In such a process, bacteria catalyze reduction reactions (by utilizing hydrogen atom and electron) of organic molecules (on the cathode) to produce a high value-added product. On the other hand, on the anode surface, the hydrogen ion is produced and passes through the exchange membrane. In fact, on the anode, under abiotic conditions, water is oxidized to protons and oxygen in the anode half reaction and the protons pass through the permeable membrane. At the cathode, the half reaction occurs by conversion of carbon sources to carbon-bearing compounds by biological film provided on the cathode. The mechanism of extracellular electron transfer is divided into the following steps: (1) direct electron transfer: nanowire, or direct contact and; (2) mediators-shuttled: [6].

Solventogenic *Clostridia* species (*C. acetobutylicum, C. beijerinckii, C. saccharobutylicum,* and *C. saccharoperbutylacetonicum)* are widely used for solvent production (acetone, butanol, and ethanol) especially butanol [7]. On the other hand, some kind of *Clostridia* such as *C. tyrobutyricum* can produce high organic acid (butyrate) using glucose [8]. Special heterotroph microorganisms like *C. pasteurianum* are able to produce biochemicals utilizing organic fermentable matters. A lot of studies have been performed to produce solvents by *C. pasteurianum* by using glycerol utilization while using glucose as a substrate has not been attended due to low

solvents production rather than organic acids production. In this study, we tried to discover that by the aid of the electricity, the more solvents especially butanol can be produced by this strain. By bioelectrosynthesis, microorganisms are able to drive electrons from the electrode for organic matter reduction. The electricity energy can be supplied by external energy resources.

Biological redox co-factor (NAD^+/NADH) plays an important role in bacterial metabolism. The ability of microorganisms to exchange electrons directly or indirectly with the cathode, and therefore drive novel reductive reactions at electrodes led to the improvement of microbial electrochemical synthesis (MECS) over the last decade [9]. In microbial metabolism, NADH is generated (NAD^+ to NADH) by oxidation reactions (e.g., 2 NADH generated from 1 glucose by glycolysis) which have to be consumed in vice versa reaction by oxidant for an appropriate redox balance [8]. The goal of this work is to maximize the butanol production during the batch fermentation process in MEC. The common glucose fermentation pathway by *C. pasteurianum* and its conversion into butanol, ethanol, butyrate along with the release of H_2 and other components is presented in Fig. 3.1. The formation of each end-product from glucose with the formation of butanol released H_2O. Therefore, to maximize the production of a specific product (butanol) in the fermentation broth, electricity supplement can be a suitable method. In fact, in this report, understanding the mechanism of bioelectrosynthesis (BES) and optimizing the operating conditions for butanol production are the main targets using glucose as a substrate and *C. pasteurianum* as a microorganism in BEC.

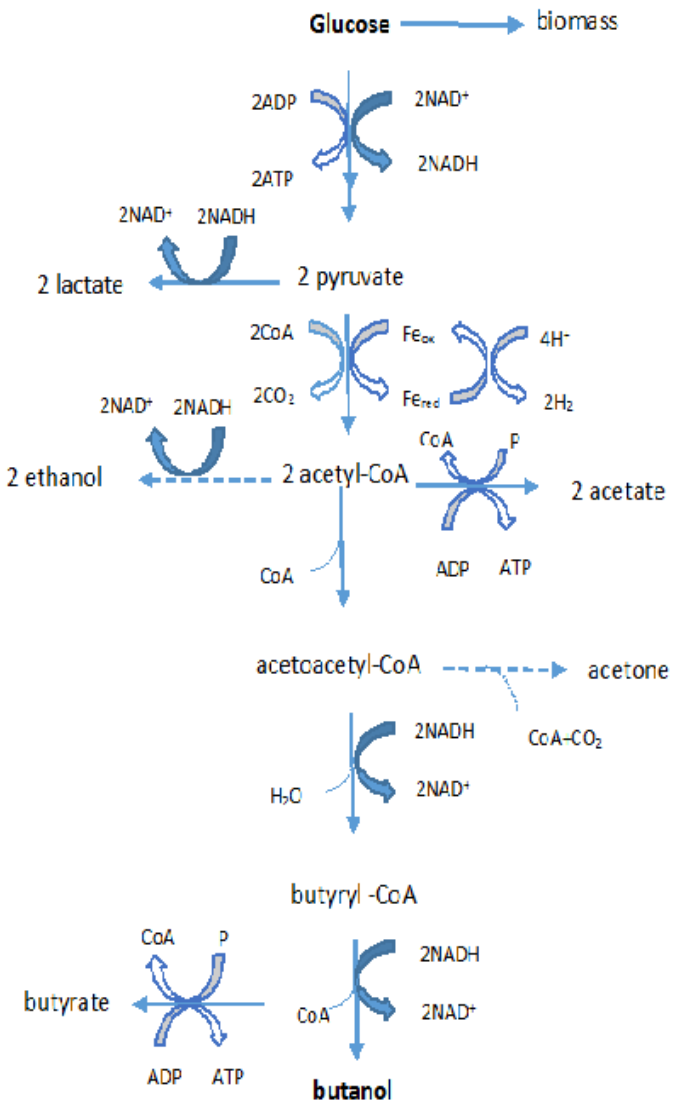


Figure 3-1 Possible metabolic pathway for glucose fermentation by *C. pasteurianum*. Dashed lines show acetone formation depending on the culture medium and microorganism strain.

3.3 Materials and Methods

3.3.1 Microorganism and medium

Choi et al [10] showed that, among *C. pasteurianum, C. acetobutylicum and C. tyrobutyricum,* just *C. pasteurianum* was the most suitable reduction peak for BES. *C. pasteurianum* ATCC 6013^{TM} was purchased from CEDARLANE (American Type Culture Collection). Freeze dried microorganism was revived and grown (after heat shock at 80 ^{o}C) in peptone-yeast extract-glucose (PYG) media under the anaerobic condition for 36 h at 37±.01 °C and 150 rpm in 125 mL serum vials (working volume 50 mL) [4]. The medium (g/L) was comprised of glucose (10); yeast extract (10); peptone (5); tryptone (5); cysteine-HCl (0.5); K_2HPO_4 (2.04); KH_2PO_4 (0.04); $FeSO_4$ -$7H_2O$ (0.0011); $CaCl_2$ (0.008); $MgSO_4$ - $7H_2O$ (0.0192); NaCl (0.08); and $NaHCO_3$ (0.4). All Chemicals were purchased from Fischer Scientific and of them were analytical grade.

Prior to fermentation, the SMM for batch cultures (fermentation) was parred in the following ingredients in 1 liter of distilled water [11]: K_2HPO_4, 1.74 g; NH_4Cl, 0.66 g; $MgSO_4$-$7H_2O$, 0.251 g; KCI, 0.596 g; Fe-Na-EDTA, 69 mg; $NaHCO_3$, 6 g; p-aminobenzoic acid, 4 mg; biotin, 0.24 mg; yeast extract, 0.5 g; resazurin, 1 mg; cysteine-HCl, 0.5 g; and glucose between 20 to 200 g. The pH was adjusted to 6.7 with KOH. MP2 medium is also tested as fermentation medium [12, 13, 14] containing (per liter of distilled water): 20 g of glucose, 0.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.01 g of $MnSO_4$-H_2O, 0.02 g of $MgSO_4$-$7H_2O$, 0.01 g of $FeSO_4$-$7H_2O$, 1 g of yeast extract, 2 g of $(NH_4)_2$-SO_4, 0.01 g of NaCl, 0.01 mg of biotin, 1 mg of thiamin, and 1 mg of p-aminobenzoic acid. The media also contained 100 mM of 2-(N-morpholino) ethanesulfonic acid (BES) to prevent over-acidification [7].

Before starting BEC, the strain was grown in 125 mL serum vials (working volume 50 mL) at pH 6.7. After sparging neutral gas for removing dissolved oxygen in the medium, the serum vial

immediately closed with aluminum crimp seal containing silicon septum (Fisher Scientific, Canada) by use of hand operated crimper (E-Z Crimper TM, VWR, Ontario, Canada). Then, it was incubated (INFORS HT) at 37 ^{o}C and 150 RPM for about 20 h to reach the exponential phase of growth. For performing experiments in MEC, the media were sterilized for 20 min at 121 °C. About 10% (v/v) of microbial culture in its exponential phase of growth (for about 20 hours and the growth rate was measured by O.D 600 (BioTek) was inoculated into cathode chamber. Anaerobic conditions were obtained by sparging the medium with CO_2 20%-N_2 80% (Linde group) for 10 min before inoculation.

3.3.2 Bioreactor setup and electro-fermentation system

Batch fermentation was carried out in a microbial electrolysis cell (Fig 3.2). Dual chamber H-type reactor (Adams & Chittenden Scientific Glass) was used consisting of two 300 mL chambers equipped with Nafion 117 cation-exchange membrane, both electrodes were graphite felt (Electrolytica, Inc Precise Town and Country) with the following characteristics: precursor material (Polyacrylonitrile fibre (PAN)), Surface area (m^2/g): 0.7, Carbon content (%): 99.7, individual fibre thickness (μ):10-20. Also, stainless steel cathode SS 304 (Rodrigue Metal Ltee Québec, Canada) was tested in the cathode chamber to compare between metallic and carbonic based materials in such a system. The space between the electrodes was 11 cm. Batch mode reactor has been widely experienced in the industry due to its simple operation and reduced risk of contamination. The power supply (PWS2000 series, Tektronix) used for linear DC electricity generation at a constant voltage. The reference electrode was Ag/AgCl 3M NaCl which was immersed in the cathode chamber. Likewise, Multimeter (Fluke-117 True RMS) was used to measure the potential between cathode and reference electrode. The reactor was immersed in a water bath equipped

with heating immersion circulators (Polystat) to operate at the constant temperature and pH. pH was continually monitored by adding KOH to the system.

Figure 3-2. Membrane dual–type bioreactor

After autoclaving the reactor, the anode compartment was filled with 0.1 potassium buffer solution (neutral pH) and cathode compartment was filled with MP2 or SMM. For preparing the medium, four solutions were prepared separately (carbon and nitrogen source contained glucose and yeast extract), potassium buffer solution (acetate buffer also can be used), vitamins, and minerals). The first solution was autoclaved at 121 ^{o}C and then cooled. The other solutions were added using filtered sterilized (0.2 µm) [15]. Fermentation was performed for 72 h at temperature 37.00±0.01 ^{0}C to initially understanding the mechanism of the system, and then at various temperatures (30-37 ^{0}C) in order to optimize the system at 150 rpm. The media were constantly agitated by a magnetic stirrer (MS-12BB, JEIO Tech Co., LTD, South Korea). The pH was adjusted by KOH at 6.7 at the initial fermentation process. High current densities can be obtained in a solution containing high buffer concentrations which leads to a lower Ohmic loss due to ionic conductivity enhancement and alleviated pH inhibition [16].

In our BES, on the anode surface, the hydrogen ions (protons) were produced (abiotic condition) and went toward the cathode through exchange membrane and in cathode compartment, microorganisms utilized electrons and hydrogen ions along with substrate in reduction reaction to produce chemicals under biotic reactions (electrodes were connected to electrical source). In contrast to the microbial fuel cell, this system utilized electrons to produce more value-added products. Simultaneously, the fermentation was performed without using electrical energy as a control system. Each experiment was performed duplicate or triplicate.

3.3.3 Analytical Methods

Liquid samples were taken regularly from cathode chamber (MEC), and control fermentation bottle separately using the sterilized syringe. The glucose as reducing sugar was measured using the DNS method including preparation of blank (1 mL water +2 mL DNS reagent) and sample (1 mL sample + 2 mL DNS reagent). After mixing well and boiling for 5 min, it was allowed to cool for 30 min. Then adding 7 mL of water, and finally taking a reading at 540 nm using Spectrophotometer (Varian). It is worth mentioning that glucose can also be measured by HPLC as well.

The butanol, ethanol, acetone, lactic acid, acetic acid and butyric acid were analyzed by gas chromatography (GC 7890B, Agilent Technologies, USA) equipped with FID detector, along with HP-INNO- Wax column (30 m, 0.25mm ID and 0.25 µm df). The initial temperature of the inlet was 50 °C and ramped up to 10 °C/min up to 150 °C and then 20°C/min up to 250 °C. Flow rate of sparging helium gas was 1 mL/min and total flow duration was 16 min at 11.421 psi. Prepared 0.500 mL sample was later injected and isobutanol was used as the internal standard. Prior to analyzing by GC, the samples were centrifuged (at 8000 g for 10 min) to separate biomass.

Cell dry weight (biomass) estimation was performed by taking the known volume of the sample with known absorbance (optical density), then the sample was filtered by filtration membrane (0.22 µm). The retained biomass was rinsed with distilled water, and thereafter, dried in an oven at 110 ^{0}C for about 8 h as described by Zhang et al [17]. A graph was plotted for estimation of dry biomass from optical density. Gas chromatography (Varian 3800, USA) equipped with a thermal conductivity detector (TCD) fitted with a 3 m PoraPLOT Q® column (Agilent technology, USA) was used for gas sample (hydrogen) analysis. Nitrogen gas with a flow rate of 3.5 mL/min with the injector, column temperature, and detector temperature was set at 100 ^{o}C, respectively. The volume of gas produced was converted to mmol, for the experimental temperature and atmospheric pressure.

3.3.4 Statistical analysis and central composite design

Response surface methodology was used to optimize the conditions. Temperature, pH, applied voltage, inoculum quantity, substrate concentration, and electrode material were tested to investigate the effective factors on butanol production in BEC. Glucose consumption rate and pH changes between the two fermentation systems (control and MEC). Moreover, analysis of variance (ANOVA) was performed to determine the significance of each parameter which showed that the glucose concentration, temperature, applied voltage, and type of electrode (cathode) are the most vital parameters. pH was also significant but, at constant pH 6.7, the best results were obtained. A central composite design (CCD) was applied to evaluate and determine the optimal operating conditions have shown not much difference for maximum butanol production and making correlations in terms of effective parameters. Butanol concentration was the targeted value. The un-coded values were selected as follows [low star point, low central point, center point, high

central point, high star pint]: substrate concentration in g/L [86.36, 100, 120,140, 153.64], voltage (V) [0, 0.2, 1.1, 2, 2.61], and temperature [27.61, 30, 33.5, 37, 39.39]. The range of temperature was chosen according to our literature review. The developed experimental design led to 20 experiments which were performed in duplicate or triplicate. Experiments were carried out in different substrate (glucose) concentrations, different temperature, and the various voltage potential to reach the optimal condition of butanol production at constant pH (6.7) and graphite felt as electrodes. The environment created by excessive levels of the substrate (glucose) could reduce the viability and fermentation ability of bacteria. If the substrate concentration is higher than a definite value, the product and microorganism concentrations will not rise with the additional substrate, resulting in wasting resources, and energy [19].

3.4 Results and discussion

3.4.1 Electrode and applied voltage

Generally, electrodes should have the following features: moderate-to-high conductivity, biological, chemical, and physical stability, economically valuable, and high specific surface area. For anode and cathode, carbon-based materials can be used, and for the cathode, metallic materials such as SS and Pt can also be used. The experiments were performed with both MP2 medium and SMM, however minimal medium gave the better result for butanol production as a goal of this work. Thus, the experiment started with 20 g/L glucose to investigate the effect of electrode materials and applied voltage. As depicted from fig 3.3, applied voltage up to 1.5 V is the critical voltage which can give the best results (butanol production) by graphite felt as a cathode (compared to SS), but after that, the graph declined. This happened because of low biofilm stability on cathode due to higher hydrogen production in BES as voltage increases. Therefore, it affected

biofilm stability on the cathode and resulted in reducing biochemical production. The other reason perhaps came from this fact that each reaction has a specific energy requirement, not more or less than that one. Hydrogen, as one of the product in *Clostridia* metabolism, is catalyzed by hydrogenases. It is well known that hydrogenase competes with NAD(P) reductase to oxidize the reduced ferredoxin; in another word, hydrogen production competes with butanol production in the typical fermentation. But higher hydrogen production at higher voltage was caused by reducing protons to hydrogen on the cathode due to lower biofilm stability. Using graphite and carbon-based materials as electrodes have various benefits, such as suitable cost, higher surface area (due to porosity), and stability, but they have lower mechanical strength compared to metallic electrodes. Although the mechanical strength of SS can be a positive factor, stronger biofilm generated on the surface of graphite felt is the main advantage. Fig. 3.3 shows the primary experiment to find the best range for applied voltage in the optimization step. Thus, when it was found that the applied voltage upper than 2 led to a decrease in butanol production with graphite felt, the SS electrode was not considered in the optimization stage.

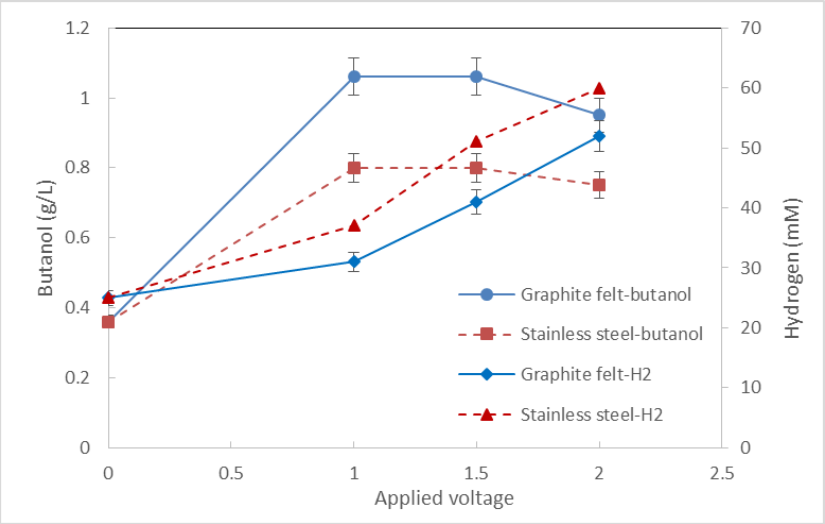
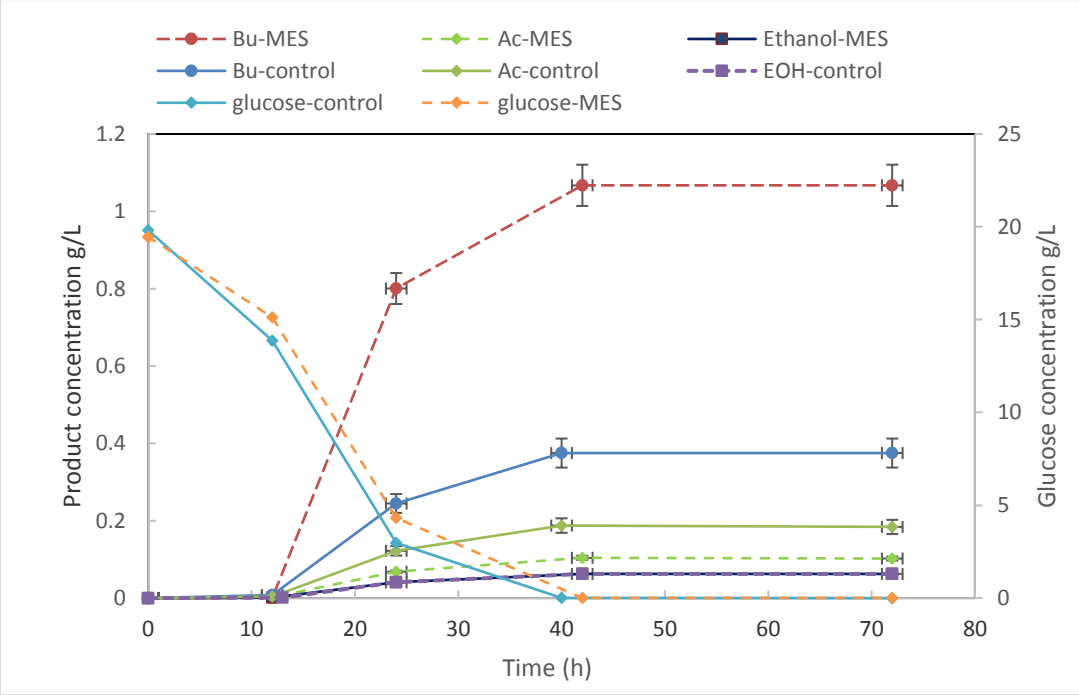


Figure 3-3 Effect of applied voltage on butanol and hydrogen production in bioelectrolysis cell (BEC) using two different cathodes. (Initial glucose 20 g/L, duration of fermentation 72 h).

3.4.2 Understanding of the mechanism of BES (Preliminary experiments)

The experiments were performed using 20 g/l glucose. Fig 3.4 (a,b) shows the results from batch fermentation at constant voltage at 1.5 V (-500 mV vs Ag/AgCl, and max 1.5 mA). Normally, pH reduced from 6.7 to 4.8 because of CO_2 and acids production, however, the higher yield was achieved at higher and constant pH 6.7, thus the experiment was performed at constant pH. The inlet glucose concentration of the bioreactor decreased over the time period. Maximum production was observed at the end of the fermentation. Glucose metabolism, butyrate, and butanol production followed oxidative and reductive pathway for *Clostridium* species. In the oxidative pathway, NAD^+ gets reduced to NADH with glucose entering glycolysis to produce pyruvate. The pyruvate, depending upon the microorganism is broken down to various products (ethanol, lactate, acetate, butyrate, and butanol) along with small hydrogen production. During the reductive pathway, NADH is reoxidized to NAD^+ with glucose finally transforming into butanol. The summarized pathway of microorganism is presented in Fig. 3.1.

a)



b)

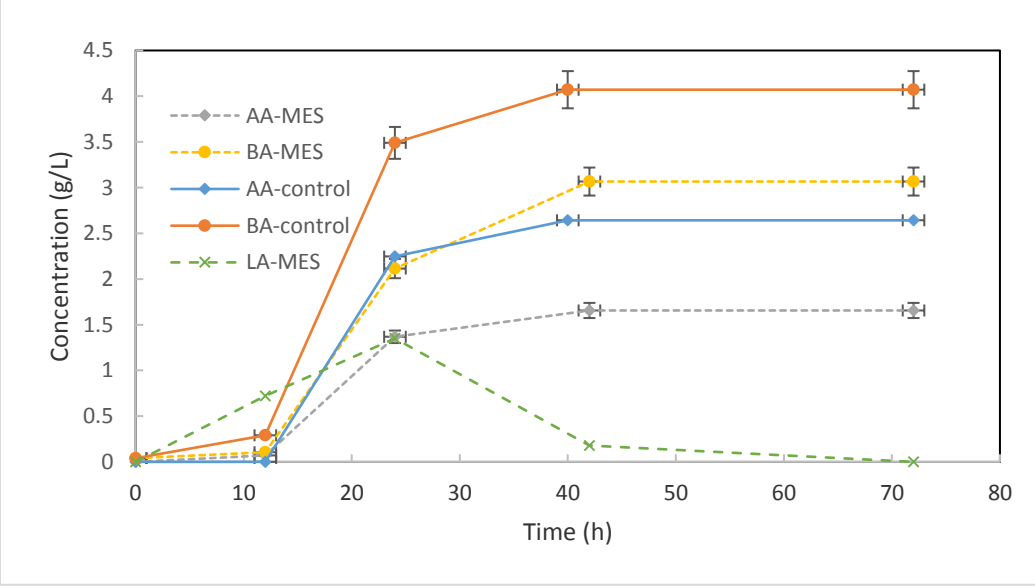


Figure 3-4 a) Total solvent production (Initial glucose 20 g/l), and b) organic acid production as a function of time during batch fermentation for control and BES. Applied voltage 1.5 V (-500 mV vs Ag/AgCl, and max 1.5 mA). AA: acetic acid; BA: Butyric acid; LA: Lactic acid; Bu: Butanol; EOH: Ethanol; Ac: Acetone;

C. pasteurianum produced higher organic fatty acids than solvent by use of glucose as substrate (Compare fig 3.4 a,b). Harris et al. [19] reported that this kind of bacterium can produce significant quantities of solvents in high sugar content medium. After 13 hours (lag phase), the chemicals were produced by *C. pasteurianum* and after about 40 hours, whole sugar was consumed. Thus, by using bioelectrolysis cell, the butanol production increased (by 260%) compared to conventional microbial fermentation (control). At the same time, butyric, and acetic acid production decreased by 25% and 37%, respectively. Consequently, organic acids are converted to solvents in bioelectrosynthetic system as a result of the metabolic shift from acidogenic to solventogenic phase. This result was achieved due to the reduction of higher NAD^+ to NADH, which led to higher butanol production. In fact, butyryl-CoA as intermediate is converted to butylaldehyde and finally to butanol. Electrons which were produced at anode transfer from the anode to cathode and the electrons were directly transferred to the strains by biofilm formation. Electron uptake from the cathode by bacterium influences intercellular redox condition by changing NADH/NAD^+ [10]. As seen previously, after a lag phase (about 13 hours), the products in the fermentation broth, increased as time passed. However, lactate appeared in MEC (see fig 3.4 b) because of the motivation by electricity and shortest route (see fig 3.1) from glucose to the product, and most probably it is converted to the butanol during the time.

Some microorganisms are able to directly accept electrons from the cathode and this mechanism has a number of potential applications [20]. While a solution inoculum was added into an electrotrophic bioreactor (the cathode plays the role as the sole electron donor), and microorganism was the potential electron acceptor, the substrate was reduced to chemical (like butanol) with a stoichiometry of electron consumption. The microbial community attached to the cathode was highly enriched compared to the control fermentation. Increasing microbes attachment on the graphite felt as a cathode in contrast to cell biofilm in the conventional system

including graphite felt without supplying electricity was observed. It is clear that the cathode and the microorganism surface have negative charges. Furthermore, it is known that electrostatic interaction (repulsion) between microorganisms and cathode is predictable, nevertheless, live biofilm formation on the cathode showed that the bacteria interacted dynamically with the cathode in the presence of the electrostatic repulsion force in MES. This fact is proof of electron accepting by bacteria in a direct way. Hence, it can be concluded that without any additional electron carrier, the improvement was achieved, thus the direct conclusion is that the direct electron transfer mechanism governs the reduction reaction.

Overall, the route of glucose fermentation by this microorganism changes from acid to solvent production (however, as butanol increased, the ethanol remained constant and acetone slightly decreased) without any severe effect on substrate consumption rate.

3.4.2.1 NADH Consumption and electron equivalent balance

BES performance is affected by some important parameters, such as microbial physiology, materials of electrodes, type of electrolytes and substrates, redox potential, reactor configuration, pH, temperature, etc. Biobutanol production is controlled by NADH limitation, four moles of NADH are consumed (by oxidation to NAD+) per each mole of butanol produced from acetyl-CoA [21].

During electrofermentation, the metabolic shifts to reduced compound production lead to the distribution of products affected by the carbon and electron flow. Thus, electron/carbon flow ration can be a decisive factor for final bioproduct contribution. The NADH-consuming butanol production led to more butanol production due to net NADH changes of -2 and 0 regarding butanol and butyrate, respectively (see also fig. 1). NAD^+ is reduced to NADH by the following reaction in BES [6]:

Equation 3-1 $$NAD^+ + H^+ + 2e^- \rightarrow NADH$$

To recognize how electron flow was changed in the metabolism of butanol fermentation in the control and BES cultures, the electron equivalent balance should be accomplished with final products. The electron donors were glucose and cathode, and 1 mol of glucose is equal to 24 e^- equivalent, as indicated in equation (8). Each half-reaction of products is listed below (equations 3-2 to 3-8). The following half-reactions can be used for electron equivalent balance (EEB) (Choi et al [10] and this work):

Ethanol

Equation 3-2 $$\frac{1}{6}CO_2 + H^+ + e^- \rightarrow \frac{1}{12}C_2H_6O + \frac{1}{4}H_2O$$

Acetone

Equation 3-3 $$\frac{3}{16}CO_2 + H^+ + e^- \rightarrow \frac{1}{16}C_3H_6O + \frac{5}{16}H_2O$$

Lactate

Equation 3-4 $$\frac{1}{4}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^- = \frac{1}{8}C_3H_5O_3^- + \frac{1}{2}H_2O$$

Butanol

Equation 3-5 $$\frac{1}{6}CO_2 + H^+ + e^- \rightarrow \frac{1}{24}C_4H_{10}O + \frac{7}{24}H_2$$

Acetate

Equation 3-6 $$\frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^- = \frac{1}{8}C_2H_3O_2^- + \frac{3}{8}H_2O$$

Butyrate

Equation 3-7 $$\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + H^+ + e^- = \frac{1}{20}C_4H_7O_2^- + \frac{7}{20}H_2O$$

Glucose

Equation 3-8 $$\frac{1}{4}CO_2 + H^+ + e^- = \frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O$$

One mole of butyrate, acetate, ethanol, acetone, and butanol are equal to 20, 8, 12, 16, and 24 e^-, equivalent, respectively. Electron distribution of substrate and products can be calculated by multiplying equivalent by moles of each compound. The bar chart (fig. 3.5) compares the products equivalence balance distributed in the final product between conventional and membrane dual-type electro-bioreactor. The electron equivalent balance of butanol was enhanced, while its value for butyrate was reduced. Meanwhile, the total electron equivalent balance increased in BES compared to the control system. The electrode (in this case cathode) can act as an electron source which leads to unbalanced fermentation. The electron transfer from the cathode can amend the medium by redox changing balance. This mechanism is able to be utilized to solve some conventional fermentation constraints. For instance, a major enhancement of the production of definite products such as 1,3-propanediol or butanol has been observed [22]. In MES, electrons from the cathode were supplied as the reducing equivalent for microbial organic matter reduction through electron transfer mechanism. The electron equivalence of butanol considerably enhanced in MEC (182%) compared to the control. The result specified that electron flow to butanol significantly raised during electrofermentation, while the electron equivalent of butyrate and acetate charged -40% and -73.9% of the electron acceptors, respectively. Thus, the electron equivalent balance showed that the more electrons were consumed to butanol production, and adversely the balance decreased for acetic and butyric acids. Totally, 14.7% increase in electron balance was observed by MES. According to this contrast, BEC systems showed favorable results relative to the control system in terms of solvent production, especially butanol. In fact, BES concept enhanced the butanol which is an objective of the proposed design and decreased organic acids

(undesired products). Therefore, this membrane bioreactor configuration can be a proposed model system for the BES process.

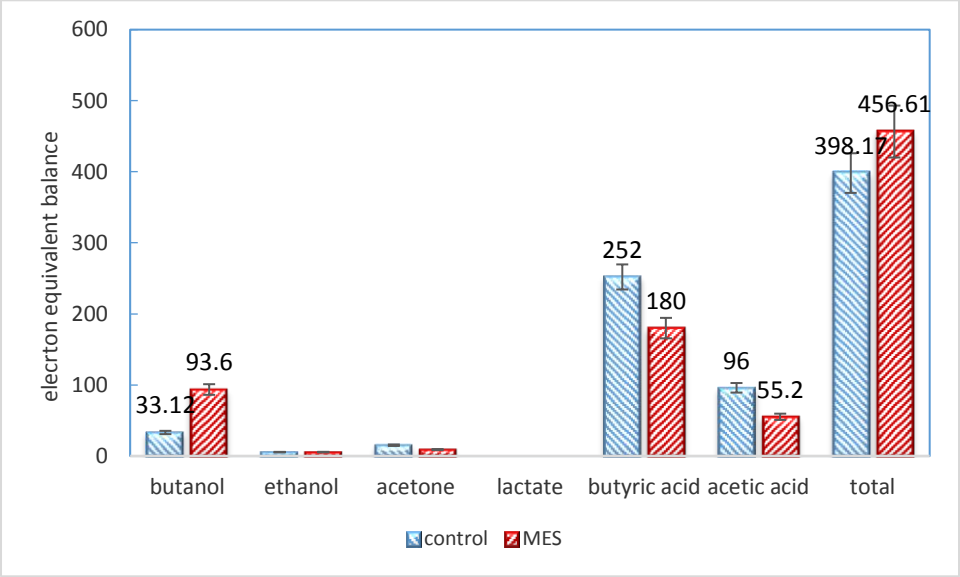


Figure 3-5Comparison of electron equivalent balance for products of control and BEC system (20 g/L glucose).

According to NADH balance (see fig 3.1), for butyrate (0), acetate (+2), butanol (-2), acetone (+2) and ethanol (0), lactate (0), the experimental results had an agreement with NADH balance. NADH consumption can be computed by the following equation [10]:

Equation 3-9 $$NADH^{cons} = \beta \Delta P$$

where β is the number of NADH required per mole of metabolism which is presented in fig 3.1, ΔP is the formation of final products in mole or mmole. The NADH consumption (as can be seen from fig. 3.6) shifted from butyrate production to butanol production in BEC.

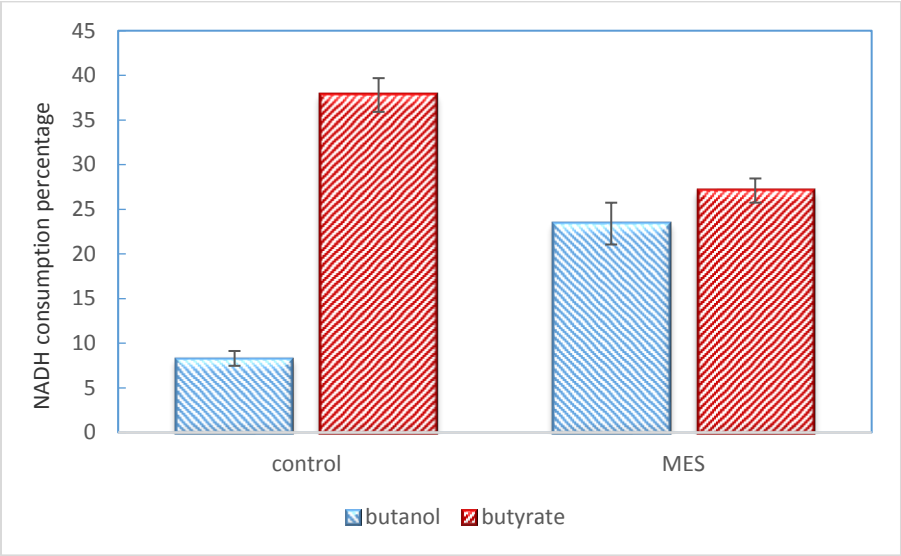


Figure 3-6 Evaluation of NADH consumption (%) by main products (20 g/L glucose concentration) for both control and BES.

He et al. [21] showed that *Clostridium beijerinckii* is a Gram-positive bacteria which has a weak capacity of extracellular electron transfer (EET) and it is not an electroactive microorganism compared to *C. pasteurianum* which is known as an electroactive bacterium [10]. Therefore the electron carriers were used, such as neutral red (NR), to enhance butanol production by *C. beijerinckii*. In comparison to *C. pasteurianum, C. beijirinkii* needs electron mediators (disadvantages) but has more productivity at lower glucose concentration (advantages). Thus, applying potential for this system just led to shorter operating time and no final product enhancement [21]. Also, He et al [19] showed that the organic acids, such as butyrate and acetate increased at the beginning of the fermentation but decreased afterwards, however, the opposite results were achieved in the present paper except for lactic acid. The results were consistent with Choi et al [10], while they did not show ethanol, and acetone concentration and they just did the experiment at constant glucose concentration, applied voltage, and one type of electrode without

examination the electron mediator addition. Moreover, previous studies on BES technology resulted in butyric acid enhancement [8], and the improvement of ethanol production [23] by BEC. As stated by Al-Shorgani et al. [24], butyric acid can be further converted to butanol by *Clostridium saccharoperbutylacetonicum*. Also, Choi et al [8] reported that butyric acid can dramatically increase in BEC by *C. acetobutyricum.*

The surface charge of the microbial cell is a significant factor for biofilm formation and attachment to graphite electrode (cathode). Zeta potential measurement of *C. pasteurianum* in BEC and control system has shown that the zeta potential value of bacteria in BEC was closer to zero (more stable biofilm) rather than grown bacteria without applying electricity which was found as an electronegative microorganism. Biofilm is a conglomerate of cells which grow on the electrode. Biofilm as a living biocatalyst will continue growing, and the consumption rate increases, as long as the substrate is available. This is limited by transferring substrate and product in and out of biofilm. Total NADH consumption in BES for butanol production indicated that metabolic shift to butanol reduction pathway was predominant with electron supplement. Throughout this period, total NADH consumption in BEC was more than the control system.

3.4.3 Optimization

3.4.3.1 Glucose concentration impact and inhibition mechanism

Optimization technique was done for obtaining maximum production of butanol. Primary experiments (section 3) discovered that electrode material, and applied voltage have the largest effects on butanol production by *C. pasteurianum* in BEC. For finding the best range of design experiment, glucose concentration assessment has to be performed in the absence of electricity supplement and in the presence of electricity. Fig 3.7 illustrates the solvent production by microbial

batch fermentation as a function of different initial glucose concentrations and using graphite felt as electrodes. For control system (without applying electricity), the butanol production enhances by increasing glucose concentration starting from 20g/l and reached the peak of 10.2 g/L butanol production (at 120 g/L glucose concentration), while afterwards, it declined and some amount of glucose remained at the end of fermentation. Total solvent production was 16.21 g/L at 120 g/L glucose concentration. Thus, solvent productivity was dependent upon glucose concentration in the media. As can be seen from fig. 3.7, the concentration range from 100 to 140 g/L was chosen for process optimization. From this figure, the maximum butanol production could be also obtained at 120 g/L initial glucose concentration by use of bioelectrosynthesis (BES). The butanol concentration increased as the initial substrate raised until reaching the maximum value of 13.02 g/L. More glucose concentration led to slightly decrease in solvent production as well (140 g/L). This phenomenon occurred by-product toxicity or substrate inhibition effect on microbial metabolism. Also, at higher glucose concentration rate, lag phase also increased. The time of lag phase supposed to increase as initial glucose concentration increases due to the osmotic pressure gradient between cell inside and outside environment of bacteria (broth). The lower amount of fermentation products at a relatively lower initial reducing sugar concentration can be attributed to the lower amount of carbon source.

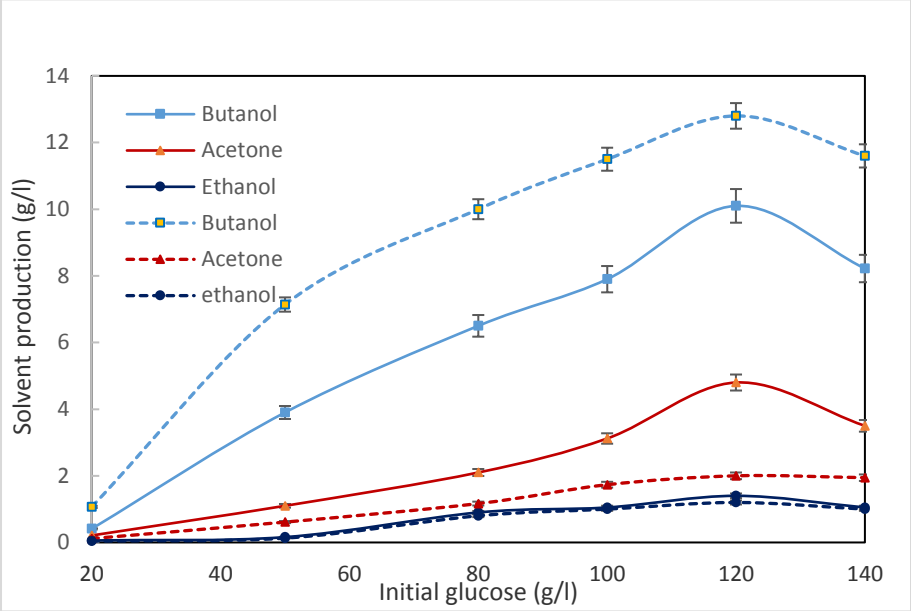


Figure 3-7Solvent (acetone, butanol, ethanol) production during batch fermentation as a function of substrate concentration, solid lines show the results for control and dashed lines show BES poised at 1.0 V (-500 mV vs Ag/AgCl, and 1.5 mA)

Table 3.1 presents the solvent yield (gram of solvent production per gram of glucose consumption) and productivity which follows the same trend in fig 3.7. From this table, the maximum yield percentage 10.1 of butanol could be achieved by batch fermentation in control and 13.03 In BEC. The productivity of acids decreased to roughly 45% as compared to the control; and the butanol productivity increased more than two-fold approximately at lower substrate concentration to around 130 % at higher sugar concentration, perhaps due to the more concentrated broth which constrains the electron transfer mechanism (see equation 3.9). Table 3.1 also shows the yield of biobutanol production during batch fermentation of conventional and microbial electrolysis cell. For instance, the butanol production increased (2.05 vs 5.34) at 20 g/ L glucose concentration, and the butyric acid decreased (20.54 vs 15.76) (not shown). This revealed that the electron uptake by *C.*

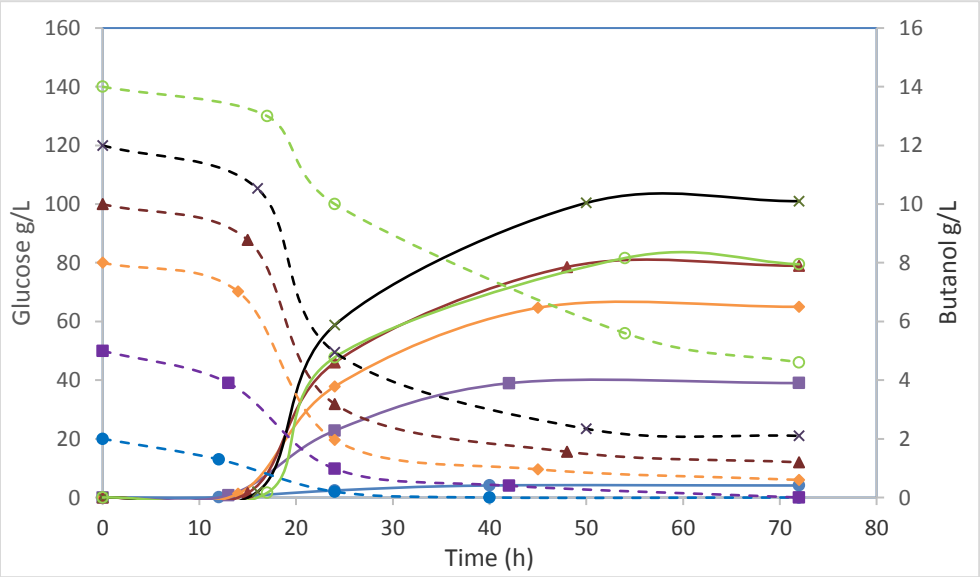
pasteurianum in BES led to a metabolic shift to consuming the reducing equivalent (NADH) by producing butanol rather than organic acids.

Table 3-1Butanol yield (g solvent/g consumed glucose) and productivity in various glucose concentration (for conventional fermentation and bioelectrochemical system) at 1.0 voltage (1.5 mA current, -500 mV vs Ag/Ag/Cl). All data were taken in duplicate or triplicate with standard deviation.

Control & BES	Control	BES	Control	BES
Initial glucose concentration (g/l)	Butanol yield (g/g) %	Butanol yield (g/g) %	Butanol productivity (g/L.h)	Butanol productivity (g/L.h)
20	2.05±0.02	5.34±0.02	0.01±0.0	0.03±0.0
50	7.80±0.1	11.80±0.1	0.09±0.01	0.14±0.01
80	8.78±0.1	12.33±0.1	0.14±0.02	0.20±0.02
100	8.98±0.2	12.78±0.2	0.16±0.02	0.24±0.02
120	10.10±0.2	13.03±0.5	0.20±0.01	0.26±0.02
140	8.74±0.2	12.13±.04	0.15±.03	0.21±0.03

To investigate the inhibition phenomenon, fig 3.8 (a,b) shows the behaviour of glucose consumption and butanol production over time for control and BES. It is obvious that at higher glucose concentration (especially after 120 g/L glucose), the final substrate concentration cannot be reached to zero unless over a very long time. The glucose concentration decreased, but after certain operation time, the glucose content remained unchanged. The other result demonstrated that the consumption of sugar was almost the same as the amount of its consumption in BES.

a)



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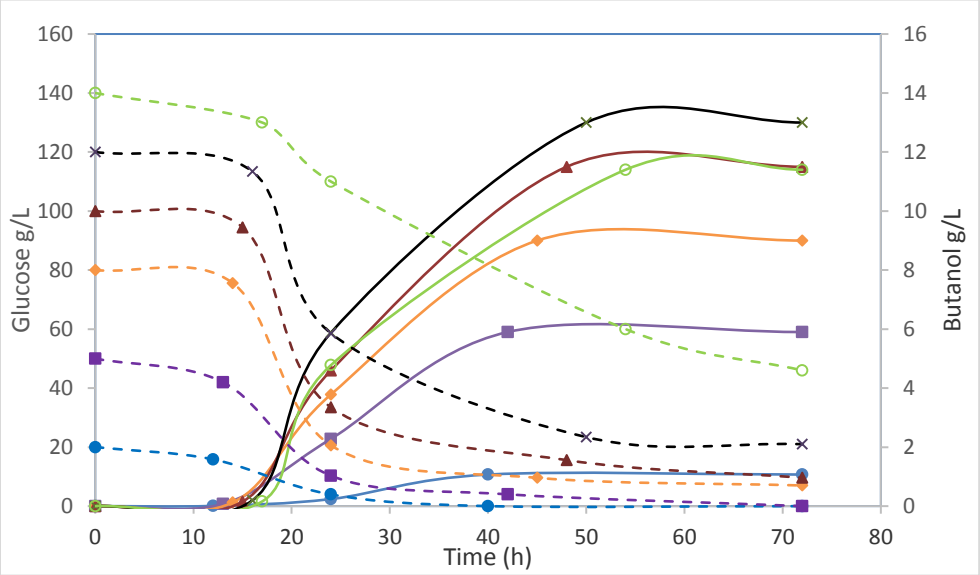


Figure 3-8a) Substrate and butanol trajectories at different initial glucose concentration (IGC) as a function of time for the control system; b) for BES. Dashed lines show glucose concentration, and the solid line shows butanol concentration. ● butanol (by 20 g/L IGC), ■ (50 g/L IGC), ♦ (80 g/L IGC),▲(100 g/L IGC), × (120 g/L IGC), and ○ (140 g/L IGC).

Specific growth rates were determined for every initial substrate concentration by correlation of logarithmic biomass (*X*) versus time (results not shown). The critical glucose concentration, maximum specific growth rate, the critical concentration of the inhibitory product, and constants were determined by an optimization technique. Bacterial growth was described by Eq. (3-10). Besides, the specific growth rate can be taken into consideration by simple Monod traditional equation (3-11).

Equation 3-10 $$\frac{dX}{dt} = \mu X$$

Equation 3-11 $$\mu = \mu_{max} \frac{S}{S+K_s}$$

An extended Monod equation can be used to study the inhibition mechanism as follows [25]:

Equation 3-12 $$\mu = \mu_{max} \frac{S}{S+K_s} \left(1 - \frac{S}{S^*}\right)^n \left(1 - \frac{P}{P^*}\right)^m$$

3.4.3.1.1 Parameter estimation and correlation

The coefficients and parameters were determined using the optimization method. Parameter estimation was used to determine the values of the unknown parameters (*a* is exponent of inhibitory product, K_s is Monod or substrate saturation constant (g/L), P^* is a critical concentration of inhibitory product above which cells do not grow (g/L), S^* is critical glucose concentration above which cells do not grow (g/L), *m, n* are power constant coefficients) achieved from the experimental data by the mathematical model. The objective function is defined as the sum of square differences (least square method) of the experimental and predicted sugar concentrations. The objective function is defined by Eq (3-13):

Equation 3-13 $$OF = min \sum_{i=1}^{n} \sum_{j=1}^{m} \left(\mu_{exp} - \mu_{cal}\right)^2$$

The parameters are given in table 3.2. A bacteria could tolerate up to about 205-210 initial glucose concentration, but the product yield and productivity substantially reduced. The maximum specific growth rate, power values of the model, and maximum butanol tolerance can be seen from the table. The too low power of product inhibition term (*m*) compared to substrate inhibition term (*n*) demonstrated that the main mechanism of inhibition is substrate concentration which simple Monod equation could not predict, however, the modified model could predict it. At a higher butanol concentration (more than 17 g/L), the suppression was predominant according to the model results.

Table 3-2Values of the specific growth rate for modified Monod model (equation 12) for control and BES.

Mode of operation	*P**	*S**	μ_{max}	*n*	*Ks*	*m*
Control	17.3	205	0.48	0.47	28.47	0.03
BES	17.4	210	0.46	0.53	26.88	0.04

The specific growth rate as a function of initial substrate concentration is depicted in (supplementary figure). The specific growth rate increased from 0.18 h^{-1} to 0.25 h^{-1} before declining. The decrease in specific growth rate (μ) at above initial substrate concentration (more than 140 g L^{-1}) was due to the presence of substrate inhibition kinetics. The cell growth was restrained in the MEC compared to the control system. The maximum growth rate was slightly lower than those in the control. The formation of biofilm on the cathode in BES could be a probable response for the low specific growth rate values of suspended cultures. Furthermore, ATP affects

cell growth. For fermentation, the only source of ATP is through the production of acetic and butyric acid besides the glycolytic route, thus, the ATP affected the cell growth slightly [10, 21, and 26].

Table 3-3 The results of the experimental design

Run	Glucose (g/L)	Voltage (V)	T (ºC)	Butanol (g/l)
1	120	1.1	33.5	13.2±0.2
2	120	0.0	33.5	10.2±0.1
3	120	2.6	33.5	11.7±0.2
4	140	0.2	37.0	10.2±0.1
5	120	1.1	39.4	10.3±0.1
6	120	1.1	33.5	13.2±0.2
7	100	0.2	37.0	10.5±0.1
8	153.6	1.1	33.5	8.5±0.1
9	120	1.1	27.6	9.5±0.1
10	100	2.0	37.0	11.0±0.2
11	120	1.1	33.5	13.2±0.2
12	120	1.1	33.5	13.1±0.2
13	120	1.1	33.5	13.2±0.2
14	140	2.0	37.0	9.9±0.1
15	86.3	1.1	33.5	8.9±0.1
16	140	2.0	30.0	10.8±0.1
17	100	2.0	30.0	10.9±0.1
18	100	0.2	30.0	11.0±0.2
19	120	1.1	33.5	13.2±0.2
20	140	0.2	30.0	9.9±0.2

Analyzing the twenty experimental data (table 3.3) by the quadratic model was significant (P-value 0.0017), according to the following equation (14). The mathematical model in terms of coded values [-1,+1] is as follows:

Equation 3-14 $$Y = \alpha_0 + \sum \alpha_i x_i + \sum \alpha_{ii} x_i^2 + \sum \alpha_{ij} x_i x_j$$

which results in:

Equation 3-15 $$B = 13.23 - 0.24G + 0.33V + 0.025T + 0.025GV - 0.025GT - 0.075VT - 1.41G^2 - 0.67V^2 - 0.99T^2$$

where B is butanol concentration, G is glucose concentration, T is temperature, and V is applied voltage. Likewise, α_0 represents the average value of the responses of the assays, x_i represents the coded variable (−1 or +1), α_i represents the principal effect of each factor *i* on the response, and α_{ij} represents the interaction effect between factor *i* and factor *j* on the response. The coefficients of the model were calculated using the half-difference between the arithmetic average of the response values when the associated coded variable is at a level (+1) and the arithmetic average of the response values when the associated coded variable is at level (−1). The coefficients show that the temperature in the given range has the least effect and the applied voltage has the highest effect on the efficiency of the system.

Table 3.4 gives information about the optimum operating condition and maximum butanol production which notably increases in BES.

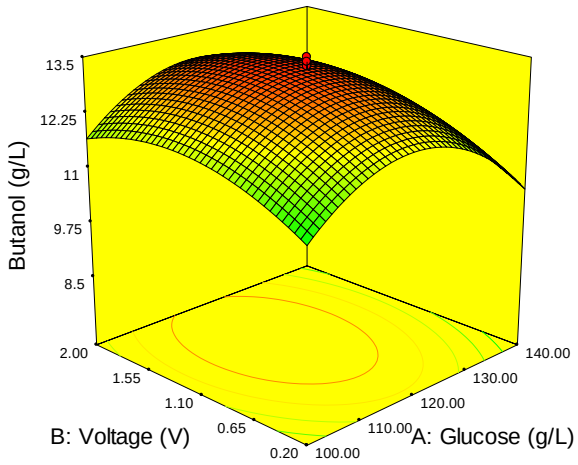
Table 3-4 Optimum conditions (by CCD) for butanol production in batch fermentation by BES.

Initial Glucose (g/L)	Temperature (°C)	Applied Voltage (V)	Butanol (g/L)	
			Predicted	Experimental
118.33	33.51	1.32 (-540 mV vs Ag/AgCl), 2.01 mA	13.27	13.31±0.2

The surface 3D plot of the combined parameters of temperature, initial glucose concentration, and applied voltage at a constant pH of 6.7 of butanol production are shown in Fig. 3.9 a/b/c. The plots

clearly indicate that an optimum exists within the observed design space with respect to three mentioned parameters appears to increase butanol yield over the observed design space.

a)



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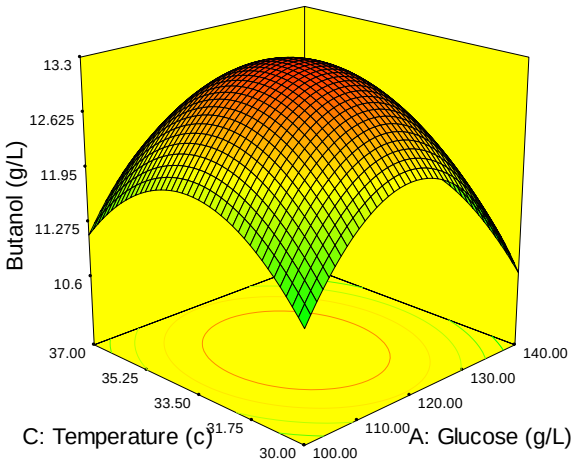

c)

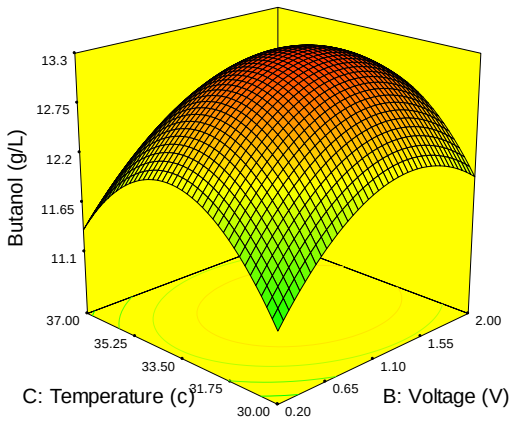

Figure 3-9 Surface 3D plot of integrated variables on butanol production at optimum condition (see table 3.3), a) voltage and glucose, b) temperature and glucose, and c) temperature and voltage.

Separation of product in fermentation processes especially butanol production involves the main energy consumption of the whole process, and researchers tried to improve this step of the process. Briefly, the separation process includes separation of biogases (H_2 and CO_2), absorption of biogases including products in order to recover and send to distillation system, broth storage tank, continue distillation, waste mesh separation, raw product separation and after that fractionation into pure products [27]. A biobutanol fermentation process leads to dilute broth and separation of the products is one of the major challenges in industrial scale. For instance, for broth purification using distillation, by increasing butanol concentration from 10 to 40 g/l in the feed, the ratio of fuel consumption reduces up to 6 times. Some useful suggestions to overcome this problem are in-situ product recovery, strain improvement, optimization the distillation process, integration of gas stripping and distillation process, coupling liquid-liquid extraction with distillation [28] and microbial electrosynthesis.

The real industrial or agricultural substrates (such as brewery liquid waste, starch industry wastewater, and apple pomace must be pretreated prior to fermentation in conventional or microbial electrolysis cells using diluted H_2SO_4 in order to increase the reducing sugar. Moreover, pH should be controlled to remove the excess metal ions and decrease the effect of inhibitors [29]. Large-scale fuel production is able to convert electricity into covalent carbon bonds allowing storing and transporting within the current infrastructure. If microbial electrosynthesis can be integrated with photovoltaics on an industrial scale, this technology makes it possible to create photosynthesis which has significant benefits compared to the microbial electrosynthesis which directly produces chemicals rather than biomass which needs additional processing, forming surplus waste [20]. When organic waste streams are used as starting materials in MES, the cost can be adversely affected by higher downstream processing costs because of lower conversion efficiencies and the existence of impurities and undesired products. Some downstream processes

(in addition of conventional distillation) could probably be used for MES products including in situ product recoveries techniques, such as crystallization, electrodialysis, membrane filtration, and electrochemically induced co-crystallization [30]. However, working with industrial waste has to be performed to understand the role of inhibitors completely in MEC. Some process also should be added to the conventional process such as evaporation step before feeding to the MEC in order to concentrate real industrial waste according to the optimized condition.

3.4.4 Electron mediator

Direct electron transfer requires electrochemically activity of microorganism on biocathodes. Almost bacteria were stated to be Gram-negative, whereas most Gram-positive microorganisms were not shown a robust capacity for extracellular electron transfer [31]. Therefore, some electron carriers have been used in MECs to facilitate electron transfer. NR is well known as the electron carrier in BES which is a molecule which can accept electrons from one matter and donate it to the other one (electron transport) [8, 21,32]. For instance, NR resulted in the metabolic pathway in butanol and ethanol fermentation [32]. Butanol production was enhanced by the addition of NR in a study by He et al. [21] as well. Four electron mediators were studied using *C. beijernkii* IB4, and among them, only NR illustrated the best result (for butanol production) and lower acetone formation. In fact, NR could be reduced in the acidogenesis phase, while it could be oxidized in the solventogenesis stage. Moreover, it was found that hydrogen produced in such a system was able to be catalyzed by hydrogenases which competes with the other products, however by adding NR, H_2 production declines.

In this process, energy is released. To prove and explore the function of BEC (electron transfer mechanism), the experimental results with NR addition (0.1 mM) were compared with potential

applying without NR at optimum operating conditions. The electron carrier was added into the BES system and the cathode potential was poised at -400 mV (vs. Ag/AgCl reference electrode), which matches the NR redox potential. The BES plus NR performance was just slightly higher than BES (Fig 3.10). This fact shows that NR cannot trigger the productivity of this fermentation system and proves that the main mechanism of electron transport is direct electron transfer through the cathode to the microbes (electroactive bacteria).

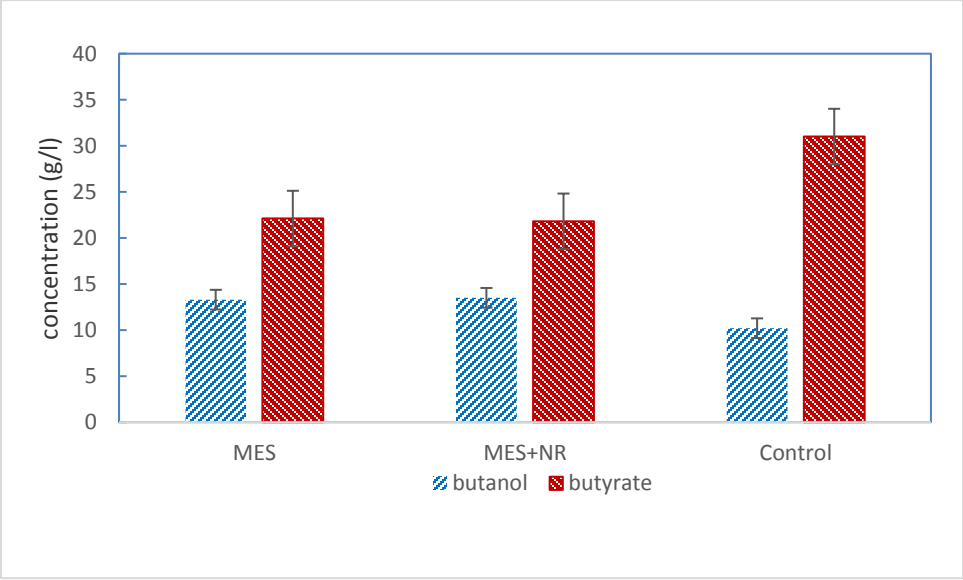

Figure 3-10 **Main product concentration in three different conditions: conventional fermentation (control), microbial electrosynthesis (MES), and MES plus neutral red (NR); Data have been shown at optimum conditions.**

3.5 Conclusion

Microbial electrosynthesis of solvents and organic acids production was performed in batch fermentation mode for 72 h. An initial experiment using 20 g/L glucose showed that *C. pasteurianum* has the potential to act as an appropriate bacteria in MEC and enhanced butanol production and reduced organic acids. Various glucose concentrations in a rage of 20-140 g/L showed that the glucose concentration higher than 120 g/l in fermentation medium led to inhibition effect by measuring specific growth rate. The cathode (graphite felt) was used as an electron donor for butanol production. The results showed that not only butanol can be increased dramatically by this technique, but also the butyric acid can be produced as a value-added product at the same time. Also, *C. pasteurianum*, is an electroactive bacterium and does not need adding electron shuttle for achieving higher performance. One potentially interesting idea for industrial butanol synthesis is using an optimal dual-type bioreactor. In this investigation, the butanol synthesis reactor was investigated and optimized (using CCD method) to maximize the butanol production rate. Overall production throughout three days of operation was considered as optimization criterion; also, three variables which were chosen as substrate amount in fermentation broth, temperature controlling (33.51 ^{0}C), and poised voltage (1.32 V) using graphite felt as electrode at constant pH (6.7) and agitation rate (150 rpm) to reach up to 13.31 g/L butanol. Microbial catalyzed electrochemical systems (MCES) can be used to remediate waste and produce value-added products simultaneously which means that apart from biochemicals production by BES, it can also be used for waste treatment and pollutant removal.

Acknowledgement

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Nomenclature

a exponent of inhibitory product

ANOVA analysis of variance

ATP Adenosine triphosphate is the energy currency of life, which is found in every cell

B butanol concentration (g/L)

BEC bio-electrolysis cell

BES bio-electrosynthesis

Bu20 20% biobutanol and 80% diesel

CCD central composite design

CI compression ignition

CO carbon monoxide

DNS dinitrosalicylic acid

EEB electron equivalent balance

EET extracellular electron transfer

EMS electro-microbial synthesis

MES microbial-electrosynthesis

ISFC indicated specific fuel consumption

K_S Monod or substrate saturation constant (g/L)

NADH Nicotinamide adenine dinucleotide (reduced form), a coenzyme found in all living cells.

NAD^+ Nicotinamide adenine dinucleotide (oxidized form),

NR neutral red

NO_x Nitrogen oxides

MECS microbial electrochemical synthesis

MP2 modified P2 medium

m, n power constants

OF objective function

P concentration of inhibitory product (g/L)

P^* critical concentration of inhibitory product above which cells do not grow (g/L)

S initial substrate (glucose) concentration (g/L)

S^* critical glucose concentration above which cells do not grow (g/L)

SMM standard minimal medium

T temperature (^oC)

t time (h)

V voltage (V)

X biomass (g)

x_i coded variable

Subscripts

$_{Exp}$ Experimental

$_{Cal}$ calculated

Greek

μ specific growth rate (h^{-1})

μ_{max} maximum specific growth rate (h^{-1})

α_0 average value of the responses of the assays

α_i principal effect of each factor *i* on the response

α_{ij} interaction effect between factor *i* and factor *j* on the response

β the number of NADH requirement per mole of metabolism

ΔP formation of final products in mole or mmole

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CHAPITRE 4

Electro-bioreactor concept for enhanced 1,3-propanediol by using electroactive microorganism and glycerol as a sole carbon source

Concept d'électro-bioréacteur pour accroître la production de 1,3-propanediol en utilisant un microorganisme électroactif et le glycérol comme unique source de carbone

Auteurs

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4 Electro-bioreactor concept for enhanced 1,3-propanediol by using electroactive microorganism and glycerol as a sole carbon source

4.1 Abstract

1,3-propanediol is widely used in the polymer industry nowadays. Electromicrobial synthesis (EMS) technology is growing nowadays due to the improvement of high value-added products. This process depends on NADH and NAD^+ and electron balances in redox reactions. Here, we produced 7.42 g/L of 1,3-propanediol (1,3-PD) by using electro-fermentation technique and *C. pasteurianum* in batch H-type reactor by use of pure and crude glycerol as carbon source using standard modified P2 medium (MP2) under the following optimum conditions: 1.6 V of applied voltage, 33.9 ^oC of temperature, and 41.3 g/L of substrate concentration (C/N ratio of 36.9 g/g). Response surface methodology (RSM) was applied to optimize operating conditions. We observed that by generating stable biofilm on graphite felt cathode, the reaction pathways go to increase in 1,3-PD production. Moreover, by electron equivalent balance and NADH calculations, we demonstrate that the 1,3-PD was enhanced in an electro-bioreactor system successfully.

Keywords: Electron equivalent balance, electro-microbial synthesis, response surface methodology, 1,3-propanediol, crude glycerol.

Résumé

En ce qui concerne ces travaux, une concentration de 7,42 g/L (contre contrôl 4.6 g/L) de 1,3-propanediol (1,3-PD) a été produite en utilisant EMS et *C. pasteurianum* dans un réacteur de type H en utilisant du glycérol pur et brut et ce, dans les conditions optimales suivantes : 1,6 V de tension appliquée, 33,9 °C de température et 41,3 g/L de concentration en substrat (rapport C/N de 36,9 g/g). Pour ce faire, la méthodologie de surface de réponse (MSR) a été appliquée en vue d'optimiser les conditions de fonctionnement. Un biofilm stable, généré à la surface de la cathode en feutre de graphite, contribuait à l'augmentation des voies réactionnelles conduisant à la production de 1,3-PD. De plus, l'amélioration de production de 1,3-PD a été démontrée avec succès dans un système d'électro-bioréacteur et ce, en tenant compte de l'équilibre d'équivalent d'électrons et des calculs NADH.

Graphical abstract

Highlights:

- Improvement of 1,3-propanediol production by microbial electrolysis cell using *C. pasteurianum*
- Crude and pure glycerol were used as substrates
- Optimization was performed by a central composite design

4.2 Introduction

Glycerol as an alternative carbon source which has recently been appealing attentions as a suitable substrate for biochemical production, since it is produced as a major byproduct of the biodiesel industry [1]. Crude glycerol can be a suitable source of carbon to convert into biochemicals such as 1,3-propanediol (1,3-PD) or butanol. It has also some advantages over simple sugar such as glucose since the reducing nature of glycerol leads to twice amount of reducing equivalent and finally higher formation of products [2]. *C. pasteurianum* is able to convert glycerol to 1,3-PD. Nowadays, 1,3-PD is used in the plastic poly-trimethylene terephthalate (PTT) which can be used for textile fibre production. Some other microorganisms can produce 1,3-PD, such as *Clostridium butyricum* and *Klebsiella pneumoniae. C. butyricum* produces 1,3-PD, butyrate and acetate, however, *C. pasteurianum* produces butanol, 1,3-PD, and ethanol as key products [3]. *C. pasteurianum* is one of the most electroactive bacteria which can be utilized in Electro-bioreactor (EBR) [4]. Conventional fermentation suffers several disadvantages. One of the main drawbacks is low productivity which can be enhanced by electromicrobial synthesis (EMS). In this process, bacteria catalyze reduction reactions by utilizing hydrogen atom and electron on the cathode to produce the desired product. Conversely, on the anode surface, the hydrogen ion is produced and passes through the exchange membrane. Under abiotic conditions, water is oxidized to protons and oxygen in the anode half reaction and the protons pass through the permeable membrane, while half cathodic reaction takes place by conversion of carbon sources by biological film provided on the cathode.

Redox co-factor (NAD^+/NADH) is a decisive factor in EMS. The capability of bacteria to exchange electrons with electrode leads to reductive reactions and subsequently the improvement of solvents and alcoholic production [5]. NADH is generated (by converting NAD^+ to NADH) which

has to be consumed in the other reactions for a suitable redox balance. In this work, mechanistic study and subsequently optimization of 1,3-propanediol production is carried out at EBR. The common glycerol fermentation pathway by *C. pasteurianum* and its conversion into butanol, 1,3-PD, ethanol, and organic acids are presented in Fig 4.1 which shows the NADH balance in details.

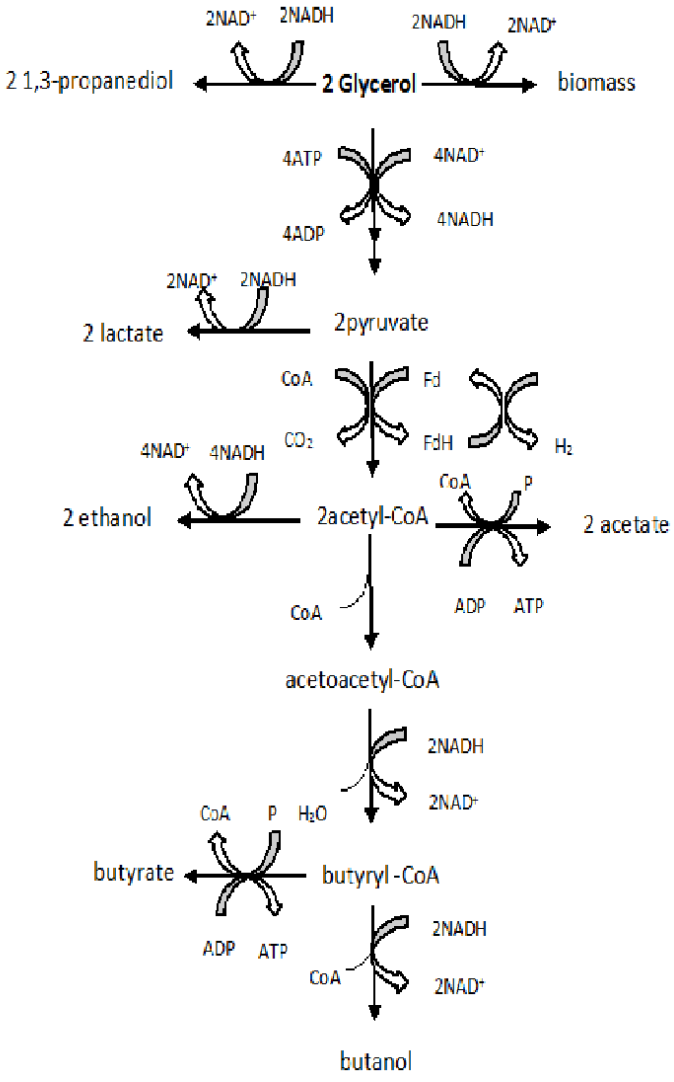


Figure 4-1 Possible metabolic pathway for glycerol fermentation by *C. pasteurianum* [1]

The ratio of carbon to nitrogen (C/N) is one of the most significant aspects in biological processes, due to microorganism's requirement of nitrogen supplementation for utilizing in metabolism during growth and fermentation steps. An optimal C/N ratio (determined by substrate concentration and nitrogen sources in broth) for solvent-producing *Clostridium* strains is essential to maximize product formation.[6] The other crucial factor is the temperature in fermentation which directly affects the bacterial growth. In EMS, the optimum applied voltage is another decisive parameter. Batch fermentation has been commonly experienced in the industry owing to simple operation and reduced risk of contamination. Thus, in the present study, the aim is to produce maximum 1,3-propanediol in MES by optimizing the effective factors such as carbon source, applied voltage, and operating temperature in the batch electro-bioreactor.

4.3 Experimental

4.3.1 Microorganism and chemicals

C. pasteurianum ATCC 6013^{TM} was procured from CEDARLANE (American Type Culture Collection). The strain was revived and grown according to our previous work [4]. All chemicals including pure glycerol were purchased from Fischer Scientific and of them were analytical grade. Moreover, the electro-fermentation has been performed in an MP2 medium which is completely described in our former study [4].

4.3.2 Bioreactor system

EMS was performed in the microbial electrolysis cell. Dual chamber H-Type reactor (Adams & Chittenden Scientific Glass) was used comprising two 300 mL chambers equipped with Nafion 117 cation-exchange membrane (Fuel Cell Store), by using graphite felt electrodes (Electrolytica, Inc Precise Town, and Country). Furthermore, stainless steel cathode (SS 304, Rodrigue Metal Ltee Québec) was verified in the cathode chamber to compare between metallic and carbon-based electrodes. The power supply (PWS2000 series, Tektronix) was used for linear DC electricity production at a constant voltage. The reference electrode was Ag/AgCl 3M NaCl immersed in the cathode compartment. Multimeter (Fluke-117 True RMS) was also used to measure the potential between cathode and reference electrode. The reactor was immersed in a water bath equipped with heating immersion circulators (Polystat) to maintain the temperature at a constant level.

The anode compartment was filled with 0.1 M potassium buffer solution (neutral pH) and the cathode compartment was filled with MP2. The media were prepared according to the procedure which has been explained in the previous study except glycerol was used instead of glucose as a substrate and carbon source. Fermentation was performed for 48 h at a temperature of 37.00±0.01 ^{0}C to initially understanding the trend of butanol and 1,3-propanediol production and then was performed at various temperatures (30-37 ^{0}C) in order to optimize the operating conditions. Two chambers were constantly stirred by a magnetic stirrer (MS-12BB, JEIO Tech Co., LTD) at 150 rpm. The pH was adjusted by KOH at 6.7 at the initial fermentation process. Higher current densities can be obtained in a solution containing high buffer concentrations which results in a lower Ohmic loss due to ionic conductivity enhancement and lessened pH inhibition. In such a system, on the anode surface, the hydrogen ions (protons) were produced at the abiotic condition and passed through exchange membrane towards cathode compartment where microorganisms utilized electrons, hydrogen ions, and glycerol to produce chemicals under biotic reactions.

Simultaneously, the fermentation was also performed in the control system (applied voltage=0) to compare with EMS. Each experiment was performed in duplicate.

4.3.3 Membrane and electrodes preparation

The electrodes and membrane were rinsed in 1N HCl and 1N NaOH, and finally was rinsed and put in autoclaved water before the starting experiments. Electrodes, fermentation medium, and all part of the reactor system were autoclaved at 121 ^{o}C for 15 min.

4.3.4 Crude Glycerol

Crude glycerol (CG) as a byproduct of biodiesel production was obtained as a gift from Rothsay, Quebec, Canada. Crude glycerol is a viscous non-homogeneous material with a gel-like appearance and a dark brownish color. A real waste, such as crude glycerol must be treated before electro-fermentation due to presenting impurities and non-fermentable chemicals. At first step, crude glycerol should be homogenized by shaking and then the crude glycerol was mixed with about double amount of deionized water for an aqueous solution (because the viscosity of crude glycerol is so high (around 0.9 Pa.s), it should be diluted for filtration). The achieved solution was then filtered via cellulosic filtered (particle retention > 25 µm-Fischer scientific) then diluted 10 fold and filtered with 0.22 µm filter to eliminate mainly fine solid particles [1]. The clear liquid was utilized for glycerol analysis and fermentation. Glycerol can be considered as an alternative to another carbon substrate due to its abundance and cost competitiveness. Furthermore, the highly reduced nature of glycerol, dissimilarly to sugars, such as glucose/xylose, leads to the production of twofold of reducing equivalents in comparison with the sugars [1].

4.3.5 Analysis by GC-MS

The treated crude glycerol (section 2.2.20 was firstly qualified using gas chromatography scan GC-MS-Thermo Scientific GC-MS model ISQ, column: DB-WAX 30m×0.2 mm i.d.×0.2 um, film thickness. By applying the column flow of 1.0 ml/min (carrier gas: helium), GC front inlet mode: Splitless, GC front inlet temperature: 225 °C, and runtime: 34.8 min. Quantifying of CG was performed using liquid chromatography (HPLC) method (Finnigan Surveyor LC Pump Plus et Autosampler Plus) by the following parameters; column: Hypersil Gold Amino 150 x 2.1mm x 3µm, oven: 35 C, flow: 0.25 mL/minute, volume injection :o10 µl, Tray temperature: 25C, mobile phase: A: H_2O 0.1% NH_4OH, D: ACN 0.1% NH_4OH, Gradient: isocratic A:15% D:85% during 11 minutes, pressure: 2200 psi.

Liquid samples were taken regularly from cathode chamber (EMC), and control fermentation bottle separately using a sterilized syringe. The products including solvents and acids were analyzed by gas chromatography (GC 7890B, Agilent Technologies, USA) equipped with FID detector, along with CP-Wax 57 CB (25 m × 250 µm × 0.2 µm). FID detector was heated to 300 ^{o}C, hydrogen, air and makeup flow was 40, 400, and 30 mL/min, respectively. The initial temperature of the inlet was 60 °C and ramped up to 20 °C/min up to 150 °C and then 40°C/min up to 225 °C (run time 10.375 min). Injection volume was 0.7 µL and the flow rate of sparging helium gas was 2 mL/min at 18.33 psi. Prepared 0.500 mL sample was later injected and isobutanol was used as the internal standard. Prior to analyzing by GC, the samples were centrifuged (at 8000 g for 10 min) or filtered (0.22 µm) to separate the biomass. Hydrogen analysis was performed by use of GC as well which was described in previous work [4].

Total nitrogen (TN) of crude glycerol was measured using Shimadzu VCPH instrument, TN Curve 0-5 mg/L method, with a detection limit of 0.02 mg/L, and total organic carbon (TOC) was

determined by NPOC Curve 0-5 mg/L method. The determination of total suspended solids (TSS), according to the analytical method (MA 115 - S.S. 1.2), was carried out by filtering a sample portion (normally 100 ml) through a previously dried and weighed Whatman 934 AH glass microfiber filter (pore size 1.5 µm) under vacuum. When the filtration was completed, the residue was dried at 103-105 °C overnight or at least 3 hours. The weight of suspended solids is obtained by calculating the difference of the filter weights before and after drying.

4.3.6 Response surface methodology (RSM)

To optimize the operating condition, response surface methodology (RSM) was used. Decisive factors, such as temperature, pH, applied voltage, substrate concentration, and electrode material were tested to explore the effects on 1,3-propanediol production in EMC. Glycerol consumption rate and pH changes were not much difference between the two fermentation systems (control and EMS). Analysis of variance (ANOVA) showed the substrate concentration, temperature, applied voltage, and type of electrode (cathode) are the most vital parameters. A central composite design (CCD) was applied to assess and determine the optimal conditions for maximum 1,3-PD production, and creating correlations in terms of effective parameters. 1,3-PD concentration was the targeted value. The un-coded values were selected as follows [low star point, low central point, center point, high central point, high star pint]: substrate concentration in g/L [9.77, 20, 35, 50, 60.23], applied voltage (V) [0.0, 1.0, 2, 2.68], and temperature (^{o}C) [27.61, 30, 33.5, 37, 39.39]. The range of parameters was chosen according to our preliminary experiments. The developed experimental design led to 20 experiments which were performed in duplicate. Experiments were carried out in different substrate (glycerol) concentrations, different temperature, and various potential to reach the optimal condition of 1,3-PD production at initial pH (6.7) by using graphite felt

as electrodes. The environment created by excessive levels of the substrate (glycerol) could reduce the viability and fermentation ability of bacteria. If the substrate concentration is higher than a definite value, the product and microorganism concentrations will not rise with the additional substrate, resulting in wasting resources and energy.

4.4 Results and discussion

4.4.1 Characteristics of crude glycerol

It was found that the concentration of methanol was too low to have any significant negative effect on glycerol utilization. The results showed that the crude glycerol contained 1160.0 g/L of glycerol, 518.0 g/L of total carbon and around 1.3 g/L of nitrogen content. Very small traces of methanol, 3-methoxy-1,2-propanediol, ethanol, 2-(2-ethoxyethoxy), 1,2,4-butanetriol, 2-piperidinone, 2-[2-(2-methoxyethoxy)], 5-O-methyl-d-gluconic acid dimethylamide, 2,4-imidazolidinedione, 5-methyl were detected. Thus, the separation step of fatty acids and soap were not needed for the samples and small traces of chemicals mostly are removed at the autoclaving step. The samples just contain pigments. The total suspended solids (TSS) was 9.5 g/l which was mostly removed by ultrafiltration (0.22 μm). In the next section, we investigate the parameters effects on glycerol fermentation.

4.4.2 Electrode and applied voltage

For anode carbon-based material, and for the cathode, carbon-based and metallic materials (SS) were used. The experiments performed with MP2 medium commenced with 30 g/L of pure glycerol to investigate the effect of electrode materials and applied voltage. Applied voltage up to

2.0 V is the critical voltage which can give the best results for 1,3-PD production by graphite felt as a cathode (compared to SS), nonetheless at voltages higher than 2.0 V the 1,3 PD dropped. This probably happened due to low biofilm stability on the cathode and higher hydrogen production (competition reaction) in EMS as voltage rises. Furthermore, each reaction has specific energy requisite, not less than that one. Higher hydrogen production at higher voltage was triggered by reducing protons to hydrogen on the cathode surface because of lower biofilm stability. Even if, the mechanical resistance of metallic cathode (such as SS) is an advantage, stronger biofilm generated on the surface of graphite felt is the main benefit. With these results, we did the preliminary experiments by using pure glycerol.

4.4.3 Preliminary experiments (general trend and mechanism)

Initially, the experiments were performed using 30 g/l of pure glycerol. Fig 4.2 (a,b) shows the results from batch fermentation at constant voltage at 1.5 V (-570 mV vs Ag/AgCl, and current of 1-2 mA). As can be seen from this figure, the 1,3-PD enhanced dramatically in EMC, however at the same time the butanol decreased compared to the control system. Moreover, ethanol enhanced slightly, while butyrate concentration remained constant approximately, but the acetate decreased drastically. Normally, pH reduced from 6.5 to 6.1 slightly. The glycerol concentration decreased over the time period. Maximum production was achieved at the end of the fermentation. Glycerol metabolism, 1,3-PD, butanol and acids productions followed oxidative and reductive pathways for *Clostridium* species. Glycerol is directly converted to 1,3-propanediol by oxidation of NADH to NAD^+. In the oxidative pathway, NAD^+ became reduced to NADH with glycerol consumption to produce pyruvate. The pyruvate, depending upon the microorganism is broken down to various products (ethanol, lactate, acetate, butyrate, and butanol) with small hydrogen production. During

the reductive pathway, NADH is reoxidized to NAD^+ transforming into butanol. The summarized pathway is presented in Fig. 4.1.

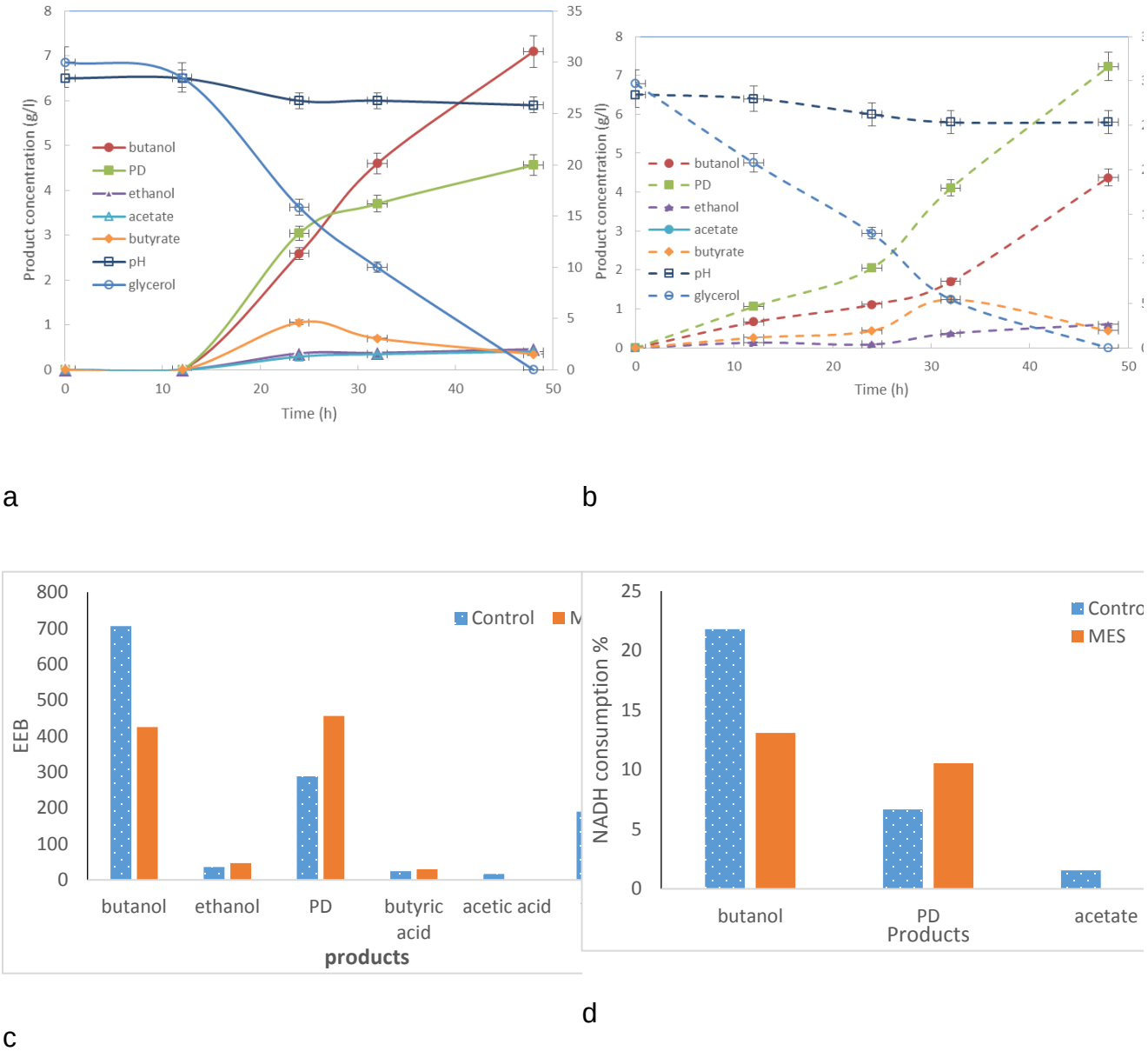


Figure 4-2Results for product formation for (a) conventional and (b) electro-fermentation of glycerol, (c) electron equivalent balance, and (d) NADH consumption at 37 ºC, initial pH 6.7, initial glycerol concentration 30 g/l, and applied voltage 1.5 V. Note the increase of 1,3-propanediol in MEC compared to control system.

After 12 hours (lag phase), the value-added chemicals were started to appear and after about 48 hours, the whole substrate was consumed. Accordingly, by using EMS, the 1,3-PD production improved (by 64%) compared to a control system (fermentation without applying electricity). Simultaneously, butanol and acetic acid production decreased by 61% and 100%, respectively. As a result, the metabolic shift happened from butanol and acetate to 1,3-PD. This result was achieved due to the reduction of higher NAD^+ to NADH, which led to higher 1,3-PD production. Electrons were transported from anode to cathode and they were directly transferred to the strains by biofilm generation on the cathode. As a matter of fact, electron uptake from the cathode by microorganism impacts redox condition by changing NADH/NAD^+.

This microorganism is able to directly accept electrons from the cathode. In this electrotrophic bioreactor, the cathode is an electron donor, and glycerol as the potential electron acceptor is converted into chemicals (such as butanol and 1,3-PD) with a stoichiometry of electron consumption. The microbial colony attached to the cathode in MES is more developed compared to the control fermentation. In spite of the presence of electrostatic repulsion between microorganisms and cathode, live biofilm formation on the cathode showed that the bacteria interacted dynamically with the cathode. This fact is evidence of electron accepting by bacteria directly.

4.4.4 Electron equivalent and NADH balances

1,3-PDl production is controlled by NADH limitation, as one mole of NADH are consumed (by oxidation to NAD+) per each mole of 1,3-PD. In the electro-fermentation system, the metabolic pathway shifts to reduced compound production results in the distribution of products impacted by the carbon and electron flow. Accordingly, electron/carbon flow ratio is a key parameter for final

product contribution. The NADH-consuming 1,3-PD production led to more 1,3-propanediol production due to net NADH changes of -1 and 0 regarding 1,3-propanediol and butanol, respectively (see also Fig. 4.1). NAD^+ is reduced to NADH by the following reaction in BES [7].

Equation 4-1 $$NAD^+ + H^+ + 2e^- \rightarrow NADH$$

To identify how electron flow was changed in the metabolism of 1,3-PD in the control and BES system, the electron equivalent balance should be accomplished. The electron donor was glycerol and cathode, and 1 mol of glycerol is equal to 14 e^- equivalent, as indicated in equation (4-3). Each half-reaction of products is listed below. The following half-reactions can be used for electron equivalent balance (EEB):

1,3-propanediol:

Equation 4-2 $$\frac{3}{16}CO_2 + H^+ + e^- \rightarrow \frac{1}{16}C_3H_8O_2 + \frac{1}{4}H_2O$$

Glycerol:

Equation 4-3 $$\frac{3}{14}CO_2 + H^+ + e^- \rightarrow \frac{1}{14}C_3H_8O_3 + \frac{3}{14}H_2O$$

The half-reactions of the other products, such as butanol, acetate, ethanol, and butyrate are described in a previous study [4].

One mole of butyrate, acetate, ethanol, 1,3-PD, and butanol is equal to 20, 8, 12, 16, and 24 electron equivalent, respectively. Electron distribution of substrate and products can be calculated by multiplying equivalent by moles of each compound. Fig 4.2 demonstrate the equivalence

balance distributed in the final product between control and MEC. As shown, the electron equivalent balance of 1,3-PD improved, while its value for butanol reduced. Furthermore, the total electron equivalent balance increased in BES compared to the control system. As a matter of fact, BEC systems exhibited favorable results relative to the control system in terms of 1,3-PD production.

According to NADH balance (see Fig 4.1), for butyrate (+2), acetate (+2), butanol (0), PD (-1) and ethanol (0), lactate (+1), the experimental results have an agreement with NADH balance. NADH consumption can be computed by the following equation:

Equation 4-4 $$NADH^{cons} = \beta \Delta P$$

where β is the number of NADH required per mole of metabolism which is presented in Fig 4.1, ΔP is formation the of final products in mole or mmole. The NADH consumption (as can be seen from Fig. 4.2) shifted from butanol and acetate production to 1,3-PD production in BEC. Total NADH consumption in BES for 1,3-PD production indicated that metabolic shift to 1,3-PD reduction pathways was predominant with electron supplement. Overall, the route of glycerol fermentation changes from positive and neutral to negative NADH balance.

Glycerol is more reduced carbon source compared to glucose (4.7 versus 4 e^- equivalent/C-substrate, respectively). *C. pasteurianum* in glycerol fermentation could produce reduced metabolites such as 1,3-PD, while it produces higher butanol by glucose fermentation due to the different metabolic pathway of a different substrate. The butanol production pathway by glucose is consuming NADH however butanol formation by glycerol is neutral NADH pathway. In fact, NADH can be directly consumed by producing 1,3-PD independently of intermediate chemicals, while by use of glucose, butanol is produced from pyruvate as NADH consumption and multiple metabolic

pathways [8]. According to the results of the experiments with pure glycerol, we performed the optimization by the use of crude glycerol.

4.4.5 Optimization

Sarchami et al [1] optimized the operating condition for maximum butanol production yield (conventional fermentation) of glycerol and crude glycerol using *C. pasteurianum* by CCD method. The results showed that a yield of 34% mole butanol per mole glycerol can be achieved at 30 ^{o}C. In this work, optimization was performed to achieve maximum production of 1,3-PD. Primary experiments revealed that electrode material and applied voltage have the major effects in the present electro-microbial cell (EMC) system. The glycerol concentration as an indicator of the C/N ratio is limited to 50 gL^{-1}. The research disclosed that the substrate inhibition of glycerol using *C. pasteurianum* is up to 170 gL^{-1}; though, it was stated that at higher 50 gL^{-1} glycerol concentration conversion is so slower [9, 10] [11]. In the fermentation medium, the substrate and the C/N ratio has a key role using solvent-producing *Clostridium*. The C/N ratios were calculated based on the percentages of carbon and nitrogen by mass in glycerol, yeast extract (1g of YE has 14 mg Nitrogen) [12] and the other nutrient in the broth.

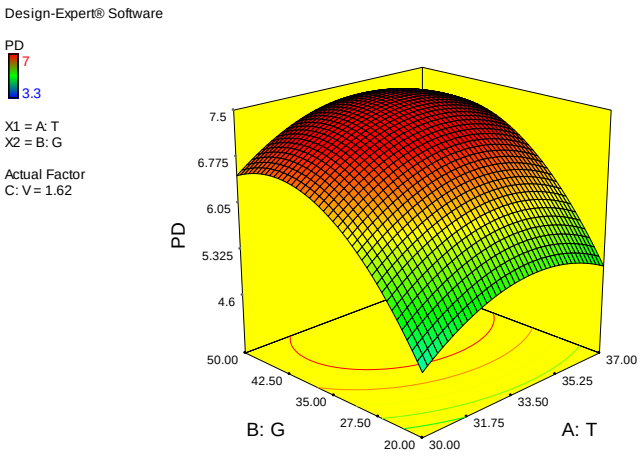
Evaluating the 20 experimental data by the quadratic model was significant (p-value < 0.0001; R-Squared=0.9782) according to the following equation (5). The mathematical model in terms of coded values (Eq 4-6) and actual values (Eq 4-7) are as follows:

Equation 4-5 $$Y = \alpha_0 + \sum \alpha_i x_i + \sum \alpha_{ii} x_i^2 + \sum \alpha_{ij} x_i x_j$$

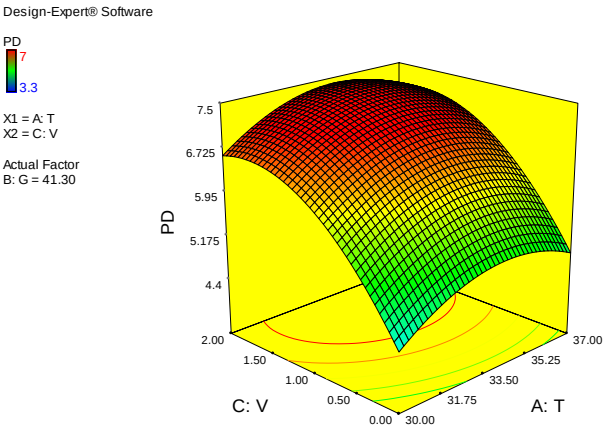
Equation 4-6 $$PD = -52.146 + 3.037T + 0.288G + 1.548V + 0.027GV - 0.0443T^2 - 0.004G^2 - 0.777V^2$$

Equation 4-7 $$PD = 6.92 + 0.23T + 0.70G + 0.93V + 0.40GV - 0.54T^2 - 0.86G^2 - 0.78V^2$$

where PD is 1,3-propanediol concentration (g/L), G is glycerol concentration (g/L), T is temperature (^oC), and V is applied voltage (V). Similarly, α_0 represents the average value of the responses of the assays, x_i represents the coded variable (−1 or +1), α_i represents the principal effect of each factor *i* on the response, and α_{ij} represents the interaction effect between factor *i* and factor *j* on the response. The coefficients of the model were computed using the half-difference between the arithmetic average of the response values when the associated coded variable is at a level (+1) and the arithmetic average of the response values when the associated coded variable is at level (−1). Table 4.1 gives information about the optimum operating condition and maximum 1,3-PD production which notably increases in BES. The surface 3D plot of the combined parameters of temperature, initial glycerol concentration, and applied voltage at a constant pH of 6.7 of 1,3-PD production are shown in Fig 4.3 a/b/c. The plots clearly indicate that an optimum exists within the observed design space with respect to three mentioned parameters appears to increase 1,3-PD yield over the observed design space. Like the previous chapter, the applied voltage has the highest and the temperature has the lowest effects on final product concentration, however, all the considered parameters are significant according to the model and ANOVA test.



a



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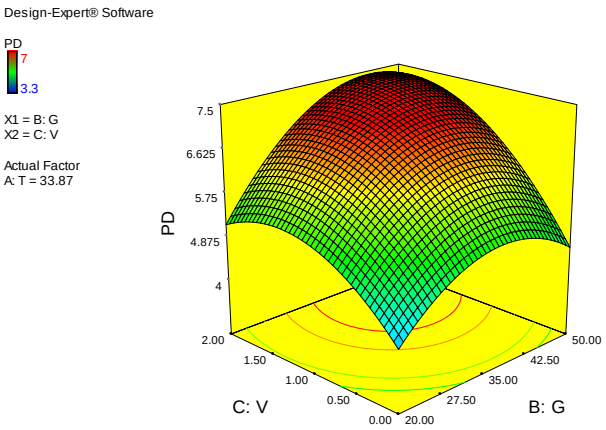

c

Figure 4-3 Optimization of 1,3-PD production as a function of operating temperature, glycerol concentration, and applied voltage; a) 1,3-PD vs glycerol concentration and temperature, b) 1,3-PD vs temperature and voltage, c) 1,3-PD vs voltage and substrate concentration. Note the maximum production of 1,3-PD on top of each curve.

Moreover, Table 4.1 presents the solvent yield (gram of solvent production per gram of glycerol consumption) and productivity for batch fermentation of conventional and microbial electrolysis cell at optimum condition. From this table, the maximum yield percentage of 11.6 of 1,3-PD could be achieved by batch fermentation in control versus 17.9 In BEC. The productivity of butanol decreased to roughly 55% as compared to the control, and the 1,3-PD productivity increased by 60% approximately. This revealed that the electron uptake by *C. pasteurianum* in BES led to a metabolic shift to consuming the reducing equivalent (NADH) by producing 1,3-PD rather than butanol.

Table 4-1 Optimum operating conditions, yield, productivity and concentration of 1,3 PD

Factor	**Name**	**Optimum level**	**Low level**	**High level**
T ºC	**Temperature**	33.87	30.00	37.00
G (g/L)	**Glycerol concentration**	41.30	20.00	50.00
V (V)	**Applied voltage**	1.62	0.00	2.00
Yield % (control) product/substrate (g/g)		11.6		
Productivity (control) (g/Lh)		0.10		
Yield % (EMS)		17.9		
Productivity (EMS)		0.16		
1,3 PD (g/L) (EMS)	**Predicted**	7.46		
	Experimental	7.32±0.11		

4.5 Conclusion

Electro-microbial synthesis (EMS) of solvents and organic acids production was performed in batch fermentation mode. An initial experiment using pure glycerol showed that *C. pasteurianum* as electroactive heterotroph microorganism has the potential to act as an appropriate bacteria in the MES system and enhanced 1,3-PD production. The cathode (graphite felt) was used as an electron donor. In this study, the MES operation was investigated and optimized (using CCD method) in a dual-type bioreactor to maximize 1,3-PD production rate by use of treated crude glycerol. Overall production throughout two days of operation was considered as optimization criterion; also, three variables were chosen as substrate amount in fermentation broth (41.3 g/l), temperature controlling (33.9), and poised voltage (1.6) using graphite felt as electrode at initial pH (6.7) and agitation rate (150 rpm) to reach up to 7.42 g/L 1,3-PD. Here we show that microbial catalyzed electrochemical systems (MCES) can be used to remediate waste such as crude glycerol and produce value-added products simultaneously which means that apart from biochemicals production by BES, it can also be used for waste treatment and pollutant removal.

Acknowledgment

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CHAPITRE 5

An insight into an electro-catalytic reactor for high value-added production from crude glycerol: optimization, product distribution and reaction pathways identifications

Un aperçu du réacteur électro-catalytique pour la production à haute valeur ajoutée à partir de glycérol brut: optimisation, distribution du produit et identification des voies de réaction

Auteurs

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5 An insight into an electro-catalytic reactor for high value-added production from crude glycerol: optimization, product distribution and reaction pathways identifications

5.1 Abstract

In this study, the electrochemical conversion of pure and crude glycerol into value-added products have been investigated. The maximum non-acidic (dihydroxyacetone/acetol/glycidol) and organic acids (acetic acid, lactic acid, formic acid) formations were optimized using response surface methodology (RSM) method in terms of electrolyte solution (pH), catalyst (electrode) type, current intensity, duration of operation, and glycerol concentration in a batch electro-catalytic reactor using platinum (Pt), platinized titanium (Pt/Ti), and black Pt (PtB). Products concentrations and distributions, and reaction mechanism and pathway have also been investigated. The results showed that under strong acidic conditions, the highest chemical production was achieved using Pt electrode, and under the optimized conditions, high acetol yield of 72% at 0.31 A during 12 hours of operation was obtained.

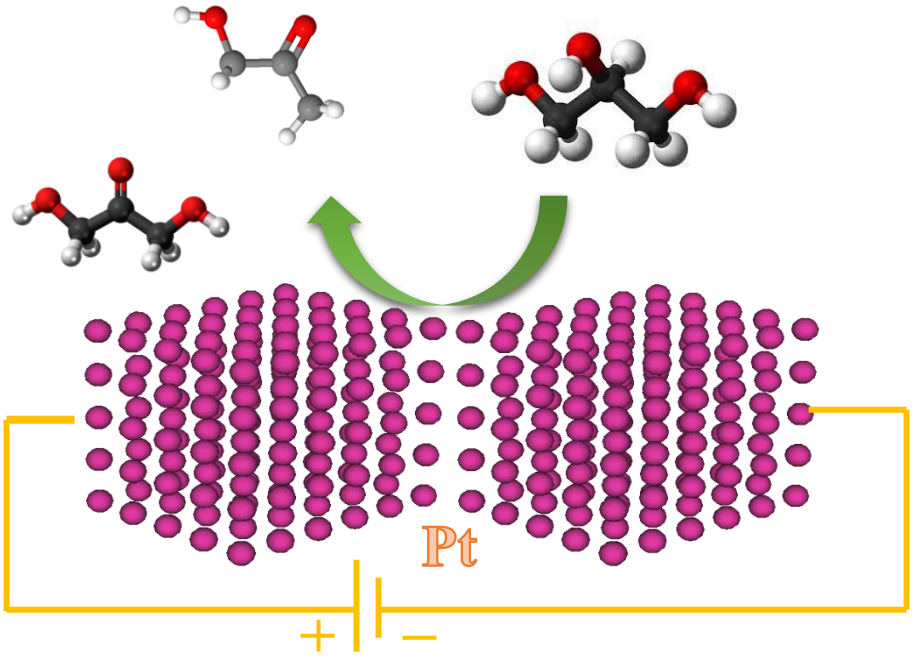
Keywords: Crude glycerol, platinum-based electrode, acidic solution, optimization, reaction route

Résumé

Dans le cadre de cette étude, la conversion électrochimique du glycérol pur et brut en produits à valeur ajoutée a été investiguée. La génération maximale de produits non-acides (dihydroxyacétone, acétol et glycidol) et d'acides organiques (acide acétique, acide lactique et acide formique) ont été optimisées par la méthode de surface réponse (RSM). Selon la chronoampérométrie et la chronopotentiométrie, le travail à courant constant a plus d'efficacité

que la tension constante. Les variables considérées étaient : le pH de la solution, le type de catalyseur (électrodes de Pt, Pt/Ti ou Pt noir, la surface de l'électrode sur le volume du réacteur, S/V = 0.13 cm^2/mL), l'intensité du courant, la durée de fonctionnement et la concentration de glycérol dans le réacteur électrocatalytique discontinu. Les concentrations et le type de produits générés ainsi que le mécanisme réactionnel ont également été étudiés. En outre, les résultats ont montré que, dans des milieux très acides (acidifié à l'HCl), une production élevée de produits non-acides a été obtenue en utilisant une électrode de Pt avec un rendement de 72% d'acétol et en imposant une intensité de courant de 0,31 A pendant 12 heures de fonctionnement.

Graphical abstract



5.2 Introduction

Nowadays, the world's capacity for biodiesel production is increasing radically. Stoichiometrically, glycerol is produced at 10 wt. % of the total biodiesel production. The present market in Asia for instance, for refined glycerol, is USD $600–650/tone, whereas crude glycerol costs USD $225–235/tone. In 2015, global biodiesel production was amplified by 13 % to 30 billion litres according to the Renewable Energy Policy Network for the 21st Century 2015 [1]. Crude glycerol in large quantities can be a potential risk to the environment. Hence, it is required to transform the crude glycerol into value-added products, which opens the new opportunities to biodiesel producers [2]. Nonetheless, the crude glycerol gained from biodiesel production has a low economic value due to the existence of major levels of various impurities, such as moisture, ash, soap, chloride, residual alcohol, traces of glycerides and vegetable pigments. Consequently, several studies have concentrated on the upgrading of glycerol from this crude glycerol, such as by simple vacuum distillation, electrodialysis, ion-exchange chromatography, and chemical and adsorption-based processes. Purified crude glycerol (96 and 99.5%) is able to be used in the food, pharmaceutical or cosmetic industries, however, this is still a restricted and saturated market. Thus, the further methodologies of converting crude glycerol to more valuable chemicals, such as synthesis gas, acrylonitrile or liquid fuels (since it is a molecule rich in functionalities with three hydroxyls (-OH) groups) are of interest. Different processes to convert glycerol into more value-added chemicals have been reported, such as pyrolysis, gasification, selective oxidation, biological processes, esterification and acetylation, and hydrolysis [3, 4]. It can also be converted to butanol, organic acids, 1,3 propanediol, or other chemicals using microbial fermentation [5]. Such processes have the most important disadvantages such as low yield and selectivity, and high cost.

Electrochemical conversion of glycerol is a novel and simple technology and is one of the methods to produce high value-added products which has not been sufficiently studied in previous reports [3]. Normally, catalytic hydrogenation reaction requires high temperatures (250–400 ^{o}C) and hydrogen pressures compared to electrochemical reductions which can occur at ambient temperature and pressure, in aqueous medium, and electrons act as reduction equivalent (while they are generated by electrode and protons are supported by protic solvents and/or by electrode) [6]. Electrochemical conversion of glycerol has been investigated due to its simplicity and vigor in terms of structure and operation. In place of using chemical reagents, this technique utilizes electrons and is then considered environmentally friendly. Electrolysis process takes place at ambient pressure and temperature and consumes less energy in comparison with chemical approaches. Moreover, electricity can be substituted by renewable resources, such as solar energy [7]. As thermochemical process point of view, controlling the selectivity and increasing the efficiency need altering temperatures and pressures, which leads to meaningfully increase in operating costs. In comparison to chemical technologies, electrochemical processes can remove the requirement of heating or pressuring equipment from the reactor system. Furthermore, it is possible to modify the selectivities by regulating the applied potentials, pH of electrolytes, and selecting the electrocatalysts to activate targeted bonds (e.g. C=C, C-H) [8].

In general, the electrochemical transformation of alcohols involves various stages: adsorption, breaking of the atomic bonds, electric charge transfer, the reaction between the oxygenated species and fragments from the alcohol, and desorption of the reaction products. Accordingly, the total efficiency depends on the interaction between the catalyst surface and the reactant molecules, interaction between the catalyst surface and the adsorbed fragments molecules, and the creation of surface oxides. As a matter of fact, glycerol conversion and the product distribution highly depend on the geometric and electronic properties of the electrodes, the

glycerol concentration, and the operating conditions of the process, such as electrolyte solution, pH, temperature, etc. [3].

Theoretically, the glycerol can be converted to the various range of chemicals such as solvents (acetol, glycidol, dihydroxyacetone (DHA), etc.) and organic acids (acetic acid, formic acid, and lactic acid). These products currently are produced through chemical or biological processes. DHA is used in cosmetics, the feedstock for the synthesis of fine chemicals, and color additive in sunless tanning products. Hydroxyacetone (acetol) is used as organic intermediates to produce polyols and acrolein, and as reduced dyes and skin tanning agent. Glycidol is applied in intermediate for the production of functional epoxides, intermediate in the production of pharmaceuticals, the additive for synthetic hydraulic fluids, and reactive diluent in some epoxy resin systems, a stabilizer for natural oils and vinyl polymers, and dye-levelling agent and a demulsifier. Acetic acid is a chemical reagent to produce vinyl acetate monomer and is used as acetic anhydride and ester, a solvent for recrystallization, and antiseptic against. Formic acid is used in the textile and leather industry, and lactic acid is mostly in the food industry [9-12]. In the past, the conversion of glycerol to formic acid was not favored due to the value competitions of them, however, by declining of the price of glycerol, this process got more attention and some studies have been done recently [13].

This work attempts to transform crude glycerol to added-value compounds by an optimized electrochemical technique. Enriched glycerol obtained by pre-treatment, can be used as the raw substrate for the electrochemical synthesis of other valuable compounds. The conversion of the crude glycerol can be optimized in terms of the solvent production to obtain the optimal product yield. According to the literature, more oxidation-reduction peaks of the treated crude glycerol were observed under very strong acid condition by Pt base electrode. Each specific product had

a different optimal applied current density and electrolysis time. Initial glycerol concentration, pH, electrode material, and current density/applied voltage are the most important factors [3, 7, 8, 14, 15].

According to the best knowledge of the authors, this is the first report on the optimization of value-added products from the electrochemical conversion of crude glycerol in the large surface area of the electrode in long time electrolysis. This work can be an opening way for scaling up this technology.

5.3 Materials and Methods

5.3.1 Optimization

In order to optimize the operating parameters, a response surface methodology (RSM) has been considered. RSM is a collection of mathematical and statistical methods for modelling, optimizing, and analyzing a process in which the response can be influenced by several variables. Central composite design (CCD) is the most widely used design in experimental design methodology. CCD allows the optimization of the process. pH, current intensity, and glycerol concentration are tested to investigate the effective factors on the production rate. Moreover, analysis of variance (ANOVA) is performed to determine the significance of each parameter which showed the mentioned factors are the most vital parameters which directly affect the product distribution and glycerol conversion. A central composite design (CCD) is applied to evaluate and determine the optimal operating conditions for maximum non-acidic production and making correlations in terms of effective parameters. Product concentration was the targeted value. The un-coded values were selected as follows [low star point, low central point, center point, high central point, and high star pint].

It is possible to determine the effect of each factor (X) as well as the effect of their interactions ($X_i X_j$) on the response (Y). This is well illustrated in the following polynomial model:

Equation 5-1 $$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

where Y is the experimental response (chemical production); b_0 represents the average value of the responses of the assays; X_i the coded variable; b_i represents the principal effect of each factor i on the response and b_{ij} represents the interaction effect between factor i and factor j on the response (Y). Design-Expert Program Software (Design Expert 7, Stat-Ease Inc., Minneapolis) is used to calculate the coefficient of the polynomial model. In order to evaluate the significance of these models, analysis of variance (ANOVA) is performed by the design expert software. A correlation coefficient that exceeds 0.8 indicates that the models are acceptable with a good correlation between predicted and experimental values. This is also confirmed by comparing Pr > F with α value (α= 0.05). Pr > F should be inferior to α in order for the models to be accepted.

5.3.2 Cyclic voltammetry study

Cyclic voltammetry is the most favorable method to investigate qualitatively information about electrochemical reactions. It can identify rapidly the redox potentials characteristic of the electroactive species [16]. To investigate the pH effect, cyclic voltammetry has been performed using a solution of pure glycerol at 3 different pH (1, 5.5, and 13) which represents the acidic, non-adjusted pH, and basic solutions. NaOH and HCl were used to adjust the pH. Moreover, in order to increase the conductivity of a solution of pure glycerol, 10 g/l Na_2SO_4 was added to the solution at pH 5.5 (pure glycerol solution without pH adjustment) to enhance the conductivity. It

was found that the most suitable electrode is platinum as the anode. The scan rate was 50 mV/s, under 500 rpm using voltalab radiometer analytical (HACH brand, ATC). The auxiliary electrode is platinum (1 cm^2). Ag/AgCl in saturated 3M KCl was used as reference electrode. A potentiostat/galvanostat (PGZ402 system, Radiometer analytical) was run by VoltaMaster 4 software (Version 7.8.26338.3, Radiometer analytical).

5.3.3 Crude Glycerol

Crude glycerol (CG) as a byproduct of biodiesel production was obtained as a gift from Rothsay, Quebec, Canada. CG is a viscous inhomogeneous material with a gel-like appearance and a dark brownish color. Real waste such as crude glycerol must be treated before electrolysis due to presenting impurities. At first step, crude glycerol should be homogenized by shaking and then the crude glycerol was mixed with about double amount of deionized water for an aqueous solution. The achieved solution was then filtered via cellulosic filtered (particle retention>25 µm-Fischer scientific) then diluted 10 fold and filtered with 0.22 µm filter to eliminate mainly fine solid particles [17]. The clear liquid was utilized for glycerol analysis and electrochemical conversion.

5.3.4 Methods of analysis

The treated crude glycerol was firstly qualified using gas chromatography scan GC-MS Thermo Scientific GC-MS model ISQ, column: DB-WAX 30m×0.2 mm i.d.×0.2 um, film thickness by applying the column flow of 1.0 ml/min (carrier gas: helium). GC front inlet mode was Splitless. GC front inlet temperature was 225 °C, and the run time was 34.8 min. Also, the qualifications of the products (before quantification) were performed by this method.

Quantifying of glycerol was performed using liquid chromatography (LC) method (Finnigan Surveyor LC Pump Plus et Autosampler Plus) by the following parameters; column: Hypersil Gold Amino 150×2.1mm×3µm, oven: 35 ^{o}C, flow: 0.25 mL/minute, volume injection:10 µl, tray temperature: 25 ^{o}C, and pressure: 2200 psi.

Liquid samples were taken regularly from the chamber. The concentration of products in the solution including acetol, DHA and glycidol were determined by liquid chromatography LC-MS-MS Thermo TSQ Quantum method, and acids were analyzed by ionic chromatography (Ion PAC AS11 ion exchange resin column 4µm) using the Thermo Integration HPLC device.

Total nitrogen (TN) of crude glycerol was measured using Shimadzu VCPH instrument, TN Curve 0-5 mg/L method, with a detection limit of 0.02 mg/L, and total organic carbon (TOC) was determined by NPOC Curve 0-5 mg/L method. The determination of total suspended solids (TSS), according to the analytical method (MA 115- S.S.1.2), was carried out by filtering a sample portion (normally 100 ml) through a previously dried and weighed Whatman 934 AH glass microfiber filter (pore size 1.5 µm) under vacuum. When the filtration was completed, the residue was dried at 103-105 °C overnight or at least 3 hours. The weight of suspended solids is obtained by calculating the difference of the filter weights before and after drying. The cations (metals) were determined by ICP-OES axial Vista Pro, power: 1.30 kW, nebulizer flow rate: 0.85 L/min, reading time per replica: 10sec.

5.3.5 Electrochemical Reactor

The experiments were conducted in a laboratory-built electrolytic cell. The cell (0.5 L capacity) were made of plexiglass (17.7×2×15.2 cm). The cathode electrodes were made of plain stainless steel and Pt/Ti mesh (11×10 cm), and the anode electrodes (11×10 cm) were

made of either mesh pure Pt (Metakem) with titanium handle, or platinized titanium Pt/Ti (mesh), or carbon-based platinum-ruthenium (Fuel cell store). The electrodes were vertically installed on a perforated Plexiglas plate placed at 2.0 cm from the bottom of the cell, and the inter-electrode gap was 1 cm. Mixing within the cell was achieved by a Teflon-covered stir bar that was installed between the perforated plate and the bottom of the cell. An electrical current was applied via a Xantrex XFR40-70 DC power supply (Aca Tmetrix, Mississauga, Canada), with a maximum current rating of 70 A at an open circuit potential of 40 V. The experiments were conducted in a batch mode. Also, the applied voltage between the working electrode and a reference electrode (Ag/AgCl) was measured by a voltmeter.

5.4 Results and discussions

5.4.1 Characteristics of crude glycerol

The production of biodiesel leads to the production of crude glycerol (CG) (10% w/w). Crude glycerol released from transesterification of fatty acids from lipids. As the biodiesel industry has grown in recent years, the availability of crude glycerol has increased. Since crude glycerol is considered as waste and non-valuable byproduct, it is necessary to develop novel techniques for utilization of the crude glycerol [18]. The impurities of crude glycerol result in its lowered price compared to pure glycerol. The concentration of the impurities varies depending on biodiesel production plants, because of differences in the feedstock, the type of catalyst used in the transesterification process, efficiency, and recovery of the methanol and catalysts. Thus, it is essential to understand the chemical composition of crude glycerol before performing

pretreatment and electrolysis. Generally, the impurities are methanol, free fatty acids (FFAs), salts, moisture, ash, soap, and methyl esters [19].

The results of our characterization showed that the crude glycerol contained 1160.0±10 g/L of glycerol, 518.0 g/L of total carbon and around 1.3 g/L of nitrogen content. It was found that the concentration of methanol was too low to have any significant negative effect on glycerol utilization. Very small traces of methanol, 3-methoxy-1,2-propanediol, ethanol, 2-(2-ethoxyethoxy), 1,2,4-butanetriol, 2-piperidinone, 2-[2-(2-methoxy ethoxy)], 5-O-methyl-d-gluconic acid dimethylamide, 2,4-imidazolidinedione, 5-methyl were detected. Thus, the separation step of fatty acids and soap were not needed for the samples. The samples just contain suspended solids pigments (pigments will automatically be removed during electrooxidation process). The total suspended solids (TSS) was 9.5±0.2 g/l which was mostly removed by ultrafiltration (0.22 µm). Table 5-1 shows the characteristics of crude glycerol in details which shows sodium and sulphur are the most abundant cations in CG.

Table 5-1 Characteristics of crude glycerol

Parameter	Concentration in Crude glycerol (mg/L)	Parameter	Concentration in Crude glycerol (mg/L)
pH	3.5	K	312
TSS (ash) g/L	9.5	Mg	18
Glycerol g/L	1160	Mn	1.36
Total organic carbon g/L	518	Mo	1.9
Nitrogen content g/l	1.3	Na	8950
F^-	0.25	Ni	30
Cl^-	0.25	P	560
Br^-	0.15	Pb	0.6
SO_4^{2-}	0.15	S	10950
NO_2^-	0.5	Sb	2.5
NO_3^-	0.1	Sc	0.07
PO_4^-	0.2	Se	2.5
Al	55	Si	19
As	1.4	Sn	0.9
B	3.8	Sr	0.55
Ba	1.5	Ti	0.2
Ca	126	Tl	1.5
Cd	0.03	V	0.2
Co	0.6	Zn	2.8
Cr	4.2	Fe	63.5
Cu	3.95		

5.4.2 Voltagrams and catalyst deactivation

Fig 5-1 shows the CV of 0.1 M pure glycerol solution under three different pHs. During the potential sweep towards increasing potentials between 0.5 V and 1 V, an anodic current peak appears at E. 0.82 V relating to the partial electrooxidation of the adsorbed glycerol. Between 0.0 V and 0.5 V only a minor current is detected, representing that the electrode

surface became saturated by the adsorbed organic compound. At a potential greater than +1.250 V, a sharp increase in the current density was observed, which is corresponded to the oxidation of H_2O to O_2. On the cathodic side of the voltammogram, the large negative current is observed, which signifies hydrogen evolution. It was found that under highly acidic condition, the current is much higher than basic or neutral conditions in the reduction area. Also, the glycerol oxidation on the Pt electrode in acidic media shows higher activity than that one in the basic media at a positive potential which has been also reported by Hunsom and Salia [3].

The stability of glycerol oxidation on the electrodes was also investigated with chronoamperometry and chronopotentiometry methods. Fig. 5-2 shows the exponential current decay caused by poisoning of the electrodes under chronoamperometry condition after around 4 h. On the other hand, under chronopotentiometry condition, the potential is increasing at the beginning of the experiments due to maintaining the current at a constant level. Current is the direct measure of the reaction rate for the electrochemical system. The reason for current decay is the deactivation of catalysts. By this fact, the experiment was performed at constant current to avoid a decrease in the efficiency of the process.

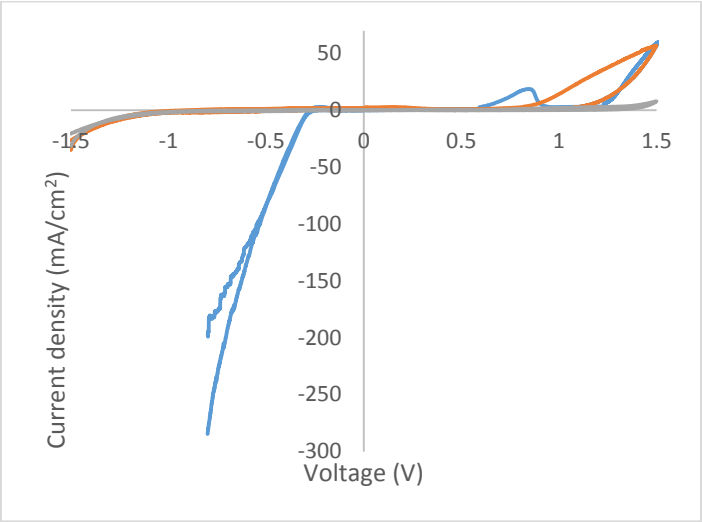


Figure 5-1**. Cyclic voltammetry of glycerol (0.1 M) at three different solutions: 0.5 M of sulfuric acid (grey line), 0.5 M sodium hydroxide (blue line), and sodium sulfate 0.1 M (red line); Pt was as working electrode (WE) and Ag/AgCl 0.3 M was the reference electrode, auxiliary electrode was Pt as well, with a sweep rate of 50 mV s^{-1}. All the WEs had an active surface area of 0.196 cm^2. The 50 ml working solution was de-aerated for at least 10 min with nitrogen gas (Linde Canada, Nitrogen Zero 0.2) to strip the dissolved oxygen.**

The anodic Pt dissolution can take place between 1.1 and 1.5 V in strong acid. The dissolved Pt was precipitated to form bigger Pt particles. Different deactivations models have been proposed such as surface oxidation, poisoning, sintering, and carbon deposition which can be mathematically modelled by linear, exponential, hyperbolic and reciprocal power, respectively [8]. The R^2 values were calculated by non-linear regressions which led to 0.78, 0.95, 0.98, and 0.76, for linear, exponential, hyperbolic and reciprocal power, respectively, which show that the irreversible adsorption of poisoning species, and Pt particle sintering, are the most overwhelming mechanism compare to metal oxide formation on the surface of catalyst and site blocking by carbonaceous species (Fig E-1).

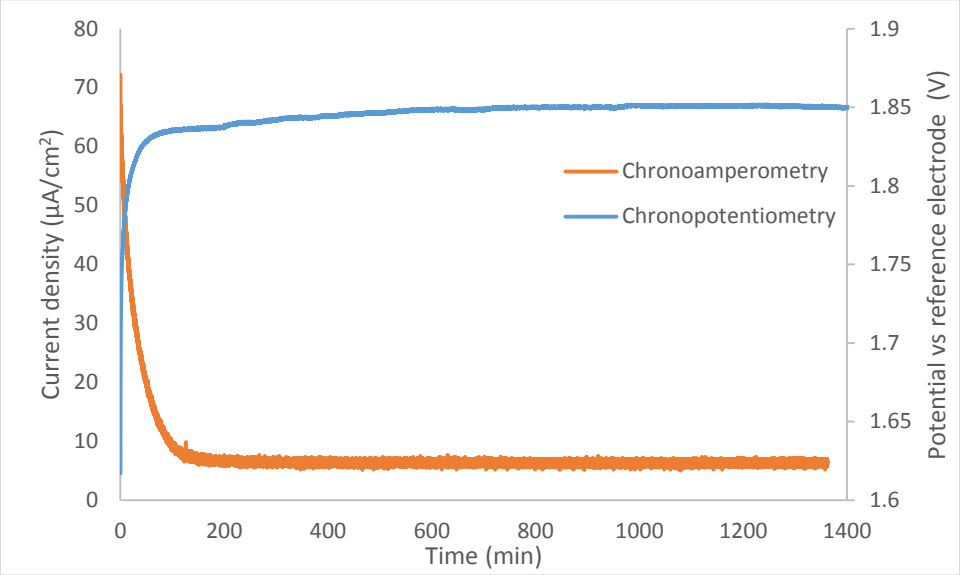


Figure 5-2. **Chronopotentiometry and chronoamperometry of a solution of 0.1 M Glycerol and 0.5 M HCl, working electrode: Pt and auxiliary electrode: Pt/Ti.**

Likewise, it is reported that the rate of glycerol oxidation is gradually decreased at higher potentials because of mass transport limitation with respect to glycerol, as the rate became limited by the diffusion of glycerol to the catalyst surface [8].

Since the current is directly related to the activity of electrocatalysts, the decline of current shows some degree of catalyst deactivation as the following reaction occurs at the surface of electrode:

Equation 5-2 $$H_2O + Pt \rightarrow Pt - OH + H^+ + e^- \qquad E^o = 0.75\ V\ vs\ RHE$$

On the surface of the electrode, glycerol is adsorbed at a higher potential (E > 0.8 V vs. RHE) which results in interaction between Pt and oxygen to form Pt-O species. Subsequently, surface oxygen will react with glycerol by dissociation of the C-C bond. Moreover, glycerol is more powerfully adsorbed because desorption or reduction of oxidized intermediates is not

thermodynamically favoured, so the remaining species will be oxidized further and leading to the production of secondary oxidation products or dissociative oxidation products [8].

5.4.3 Preliminary experiments

The conversion of glycerol into high value-added products can occur in electrochemical reactors which have several advantages. For instance, the reactions take place in aqueous media at low temperatures and pressures and the non-thermal activation leads to superior control of the selectivity. By controlling the applied current, the electrolyte pH, the glycerol concentration and operation time, and by selecting the suitable catalysts (electrode), we can modify the activity of redox reactions and the selectivity towards targeted products [20]. Preliminary tests were performed using pure glycerol to identify the best range of operating conditions and product distributions in terms of pH, current density, electrode materials, and duration of operation, and mechanistic recognition as well. It was found that pH and electrode potential during long-term potential-controlled electrolysis have the major effects on electrochemical conversion (redox) of glycerol. As expected, the glycerol conversion augmented with increasing electrolysis time and the best results (product formation) have been reached under acidic condition rather natural or basic conditions. Acetol, DHA, and glycidol as non-acidic products and formic acid, acetic acid, and lactic acid as acidic products are the main output of the experiments in the acidic solution in the applied potential range “oxygen region.” Moreover, in alkaline media (Fig E-2), the conversion rate is higher although lower products formation have been produced which demonstrates the complete oxidation (combustion) of glycerol under basic condition.

At low pH, the competition reaction of water and glycerol oxidation is occurred (E^o=1.23 V vs normal hydrogen electrode (NHE)). The reduction reaction of the components comprising the

hydroxy groups (such as glycerol) is tough because of their negative reduction potentials [6]. While the oxidation of glycerol can be performed simply at high potentials, the selective generation of specific products is challenging.

Platinum favours the oxidation of the primary alcohol group which resulting in carboxylate compounds formation. If the potentials are higher (e.g. in oxygen region), the cleavage of the C-C bond becomes dominant and the species contain C_1 and C_2 are produced. However, the high glycerol conversion and kinetic rates only correspond to the small amount of product yield; hence, glycerol might have been converted into other volatile side products, such as CO_2, which could not be detected in the liquid phase of the system. This result could be due to the high electrical energy requirement for adsorption of the OH molecule on the Pt electrode surface for electrochemical process enhancement.

As can be seen from Fig. 5-3 the glycerol conversion is decreasing by reducing the applied current which demonstrated that the rate of reaction highly depends on the applied current, for instance by reducing the current from 1 to 0.1 A (10 times) the conversion reduced about 500 %. By the fact that at lower current, the conversion is too low and at high current the conversion of glycerol is going to mostly complete combustion reactions, finding the optimum applied current is necessary.

Fig 5-4 shows that by using the Pt/Ti as the anode, the glycidol, DHA, acetol, formate, and lactate, were produced at current intensity of 0.3 A, however by increasing the current to 0.4 A the production of non-acetic compounds were reduced and no formate and lactate were observed which implies that the decomposition of adsorbed glycerol can occur during a lower applied current intensity. The glycidol is obtained from the rearrangement of the glycerol carbonium ions, derived from the protonation of the second hydroxyl group of glycerol. The non-

acidic products were rapidly produced during the first 6 hours of operation and then decreased most probably due to the second oxidation process by the Pt. For acetol, and glycidol they were generated at medium current intensity, suggesting that the dehydration of the adsorbed glycerol molecule at the first hydroxyl group is not preferred at a low or high current.

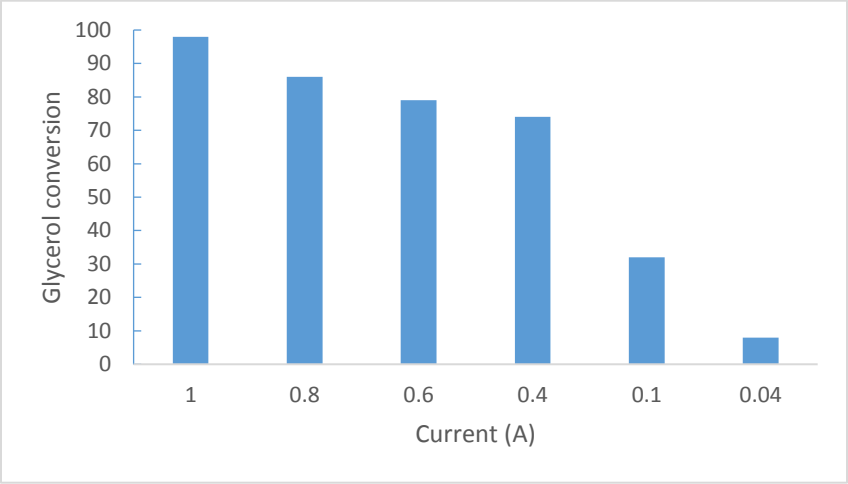


Figure 5-3. **Glycerol conversion in terms of solution media and current intensity, initial glycerol 0.3 M, under acidic condition 0.5 M, at room temperature, during 24 h. The deviation is 5% in double experiments.**

Fig 5-5a depicts by using Pt as the anode, the acetol and DHA were formed, but not the glycidol, which suggested that the pure Pt is not appropriate for rearrangement of carbonium group of glycerol. Moreover, there were just observed organic acids by increasing the current from 0.3 A to 0.4 A which shows that at lower current the simplest rout of glycerol conversion (dehydration) occurs. Fig 5-5b also shows the same experiments at higher current 0.5 and 1 A. It is observed that the acetol is not produced at higher current (both 0.5 and 1 A), but the same trend happened for DHA. Furthermore, formate, lactate, and acetate were produced more at a higher current which suggests the reaction pathway shifted from non-acidic to acidic compounds

at a higher potential. Fig 5-6 shows by applying the Pt/Ru black anode, the reaction pathway mostly went to glycerol combustion and just glycidol as the non-acidic compound was produced. At the same time, acetate, formate, and lactate were produced at relatively low concentrations.

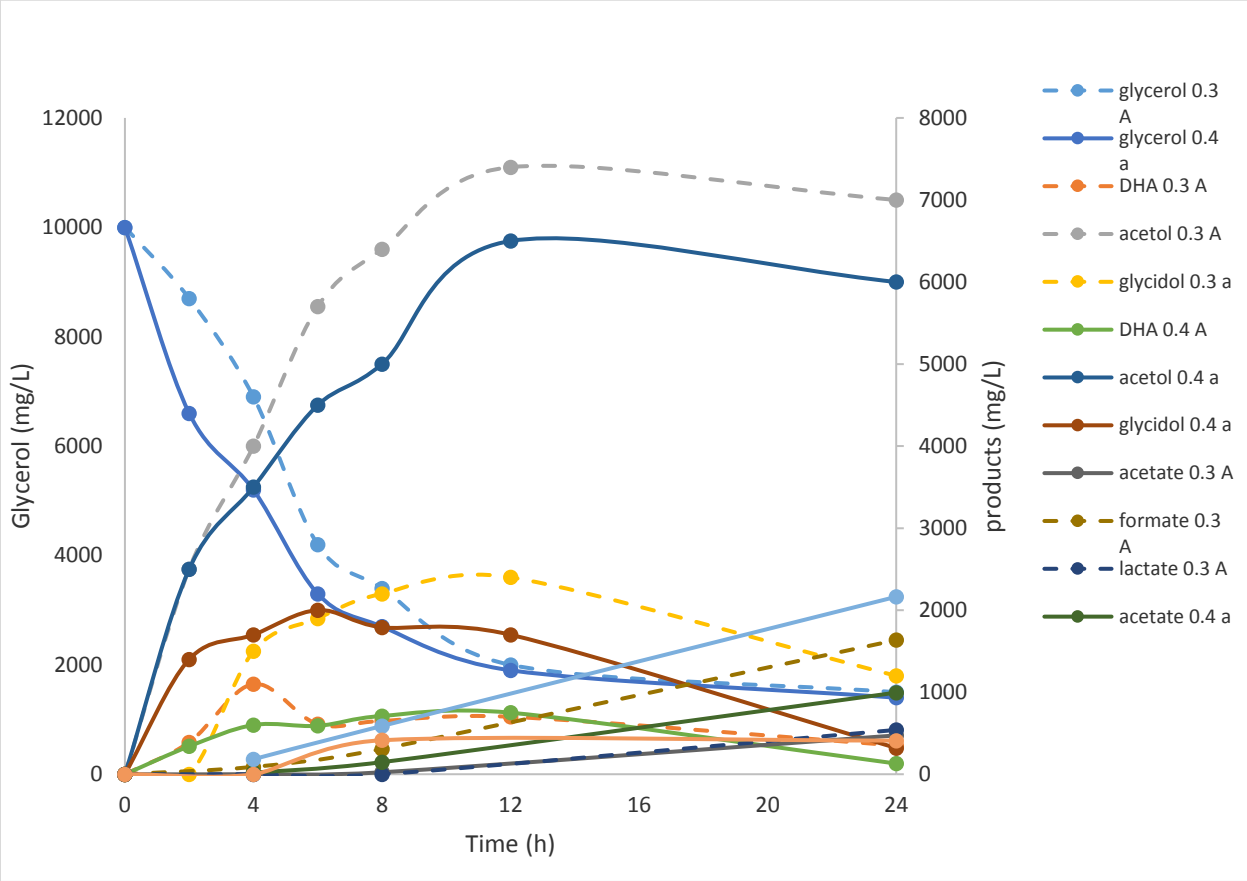
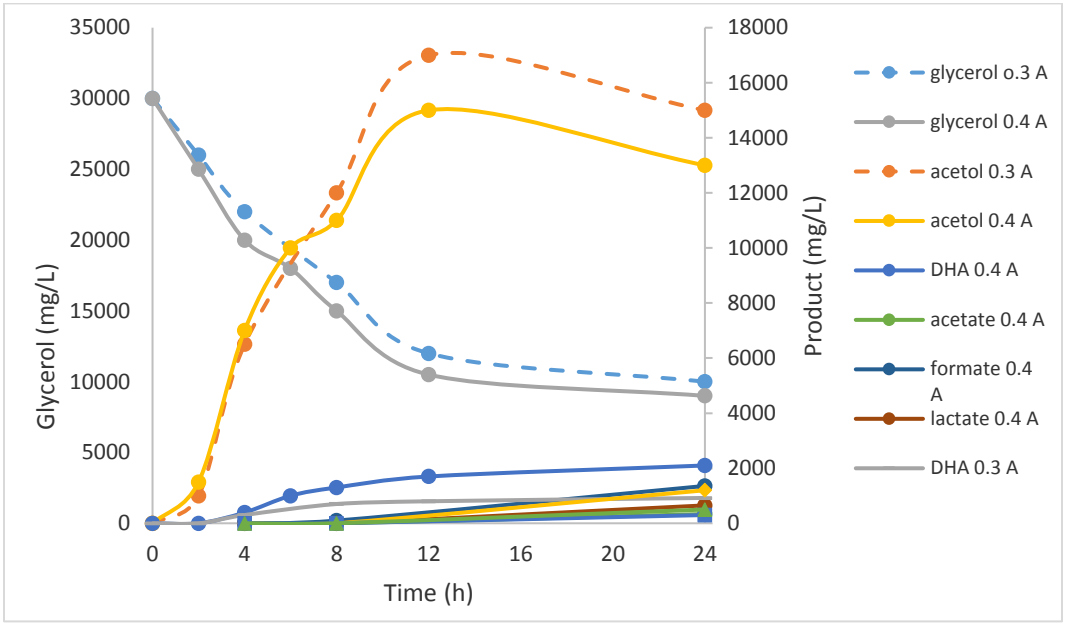
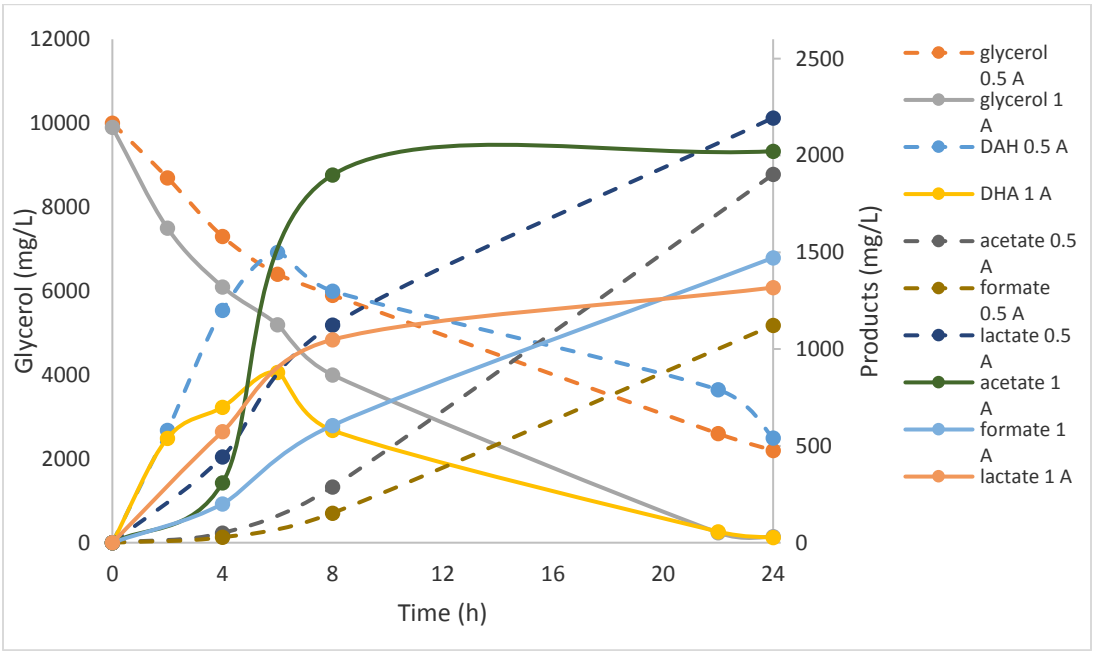


Figure 5-4. Electrocatalytic conversion of glycerol (0.1 M); anode: Pt/Ti; Cathode: SS, in acidic solution 0. 5 M HCl, constant current I=0.3 and 0.4 A. Deviation is 6% in double experiments.

Applied voltage and also the voltage between the reference electrode (Ag/Agcl; 3 M) for the glycerol electrochemical conversion by using Pt electrode is shown in Fig E-3. The applied voltage is sharply increasing at lower current intensities (from 0.1 to 0.4) and after that, it increases smoothly which suggests non-linear relation between voltage and current in this system.



a)



b)

Figure 5-5 a)Electrocatalytic conversion of glycerol 0.3 M; anode: Pt; Cathode: Pt/Ti, in acidic solution 0.5 M HCl, constant current I=0.4-0.3 A (V anode 1.8 V; V cathode: -0.4 V vs Ag/Agcl). Deviation is 6% in double experiments, b) electrocatalytic conversion of glycerol 0.1 M; anode: Pt; Cathode: Pt/Ti, in acidic solution 0.5 M HCl, constant current I=0.5-1.0 A. Deviation is 7% in double experiments.

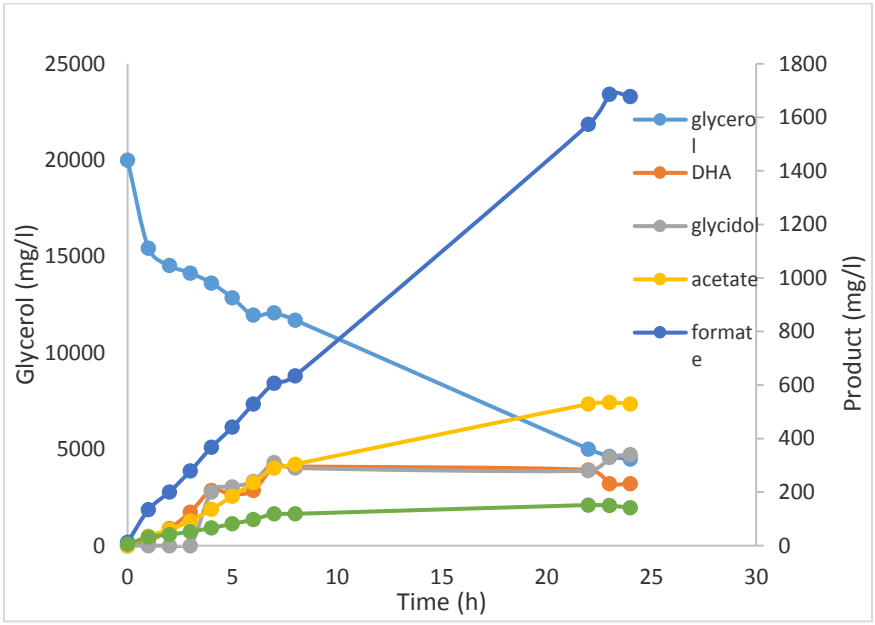


Figure 5-6 Glycerol conversion in the presence of Pt black for glycerol solution in HCl 0.5 M. 4mg/cm^2 platinum-ruthenium black carbon cloth electrode, I =0.5 A. Deviation is 6% in double experiments.

5.4.4 Kinetic model

Variation in electrode potential in the electrocatalytic reaction is connected to the Gibbs free energy. If the electrochemical process involves an adsorbed product, the electrode potential influences the Gibbs chemisorption energy of the products. Accordingly, the electrode potential control in such a system can be used to control the rates of competing for electrocatalytic reactions and the product selectivity [21]. It is also reported that glycerol electrooxidation is the first order kinetic model in similar systems [8]. The first order kinetic model is as follows:

Equation 5-3

$$C = C_0 \exp(-kt)$$

where C is glycerol concentration at time t (mg/L), C_0 is initial glycerol concentration, k is kinetic rate constant (h^{-1}), t is time (h). The glycerol conversion rates were about 40%, 69%, and 98%

at electric currents of 0.3, 0.5 and 1.0 A, respectively. The rate constant also increased from 0.029 h^{-1} (0.3 A) to 0.033 h^{-1} (0.5 A) and to 0.16 h^{-1} (1.0 A) (Fig E-4).

5.4.5 Reaction mechanism and pathway

The overall reactions can be clarified by the parallel reaction way mechanism [22]:

Equation 5-4 $$C_3H_8O_3 \rightarrow H_2 + C_n(n = 1 - 3) \rightarrow 2H^+ + 2e^- + C_n \quad (indirect)$$

Equation 5-5 $$C_3H_8O_3 + 3H_2O \rightarrow 3CO_2 + 14H^+ + 14e^- \quad (direct)$$

Equation 5-6 $$I_{total} = I_{indirect} + I_{direct}$$

The direct electro-oxidation becomes dominant at higher potentials. Thus, the compromise between applied current, glycerol conversion and product yield and selectivity is necessary. The indirect reaction pathway for H_2 oxidation implicates a mass transport and a charge transfer process due to very low H_2 concentration, while, the direct reaction route can be expressed by a charge transfer process for glycerol oxidation. In fact, the following equilibrium takes place on the Pt electrode in acidic solution:

Equation 5-7 $$PtO + H^+ + e^- \leftrightarrow PtOH$$

The adsorbed OH species on the Pt surface interact with glycerol and oxidize glycerol into products. As time passes during the operation, stable oxides are formed on the Pt surface, and the oxygen molecules can interact with glycerol molecules and make C–C bond dissociation, which results in the formation of two carbon molecules such as formate. An indirect electrooxidation pathway can occur by oxidative breakage of the C–C bond of glycerol with the hydroxyl radical (OH^o) created by adsorbed water molecule on the electrode surface, and then

by dehydration and reduction reactions. Two free radical compounds (alcohol-free radical and ethylene-free radical), are generated from C–C bond cleavage which may further dehydrated or reduced to the other compounds [3, 15].

PtOH can also be formed via the discharge of hydroxide ions:

Equation 5-8 $$Pt + OH^- \rightarrow PtOH + e^- , E = 630\, mV$$

PtOH may also become to platinum oxides [23]:

Equation 5-9 $$PtOH + OH^- \rightarrow PtO^- + H_2O$$

Equation 5-10 $$PtO^- \rightarrow PtO + e^-$$

When the products compete with the reactant for chemisorption on the active sites of the electrode, the reaction is self-inhibited which may reduce the conversion rate.

The reaction products are divided into three main categories: reduction, oxidation, and dehydration products. The dehydrogenation of glycerol (oxidation) to DHA can be demonstrated as follows [24]:

Equation 5-11 $$C_3H_8O_3 \rightarrow C_3H_6O_3 + 2H^+_{aq} + 2e^- \quad anode\ rection\ (oxidation)$$

The formation of oxidized glycerol occurs through the adsorption of the glycerol molecule on the catalyst surface as following procedure [25]:

Equation 5-12 $$Pt + C_3H_8O_3 \rightarrow Pt - C_3H_8O_{3(ad)}$$

Equation 5-13 $$Pt - C_3H_8O_{3(ad)} \rightarrow Pt - C_3H_6O_{3(ad)} + 2H^+ + 2e^-$$

Equation 5-14 $$Pt - C_3H_6O_{3(ad)} \rightarrow Pt + C_3H_6O_3$$

The dehydration of glycerol to acetol and glycidol takes place as following reactions:

Equation 5-15 $$C_3H_8O_3 \rightarrow C_3H_6O_2 + H_2O \quad Dehydration$$

Acetol can be achieved via dehydration by removing the water molecule from glycerol. The mechanistic reaction of glycerol dehydration under the acidic condition is as follow [26] [10]:

Equation 5-16 $$C_3H_8O_3 + H^+ \rightarrow (C_3H_9O_3)^+ \rightarrow C_3H_6O_2 + H_2O + H^+$$

Glycidol is obtained from the rearrangement of the glycerol carbonium ion or the cyclization, derived from the protonation of the primary –OH group of glycerol [27].

The reaction route and product selectivity can be significantly affected by the acidity of the electrolyte. As mentioned, one factor that limits the liquid product formation is the direct combustion of glycerol. Another factor might be the predominant competing reactions, such as water oxidation at the anode, a common limitation for the acidic aqueous system.

Equation 5-17 $$O_2 + 4H^+ + 4e^- \leftrightarrow 2H_2O \quad E^o = 1.23\,V\ vs\ NHE$$

Also, the hydrogen evolution reaction takes place at the cathode.

Equation 5-18 $$2H^+ + 2e^- \leftrightarrow H_2 \quad E^0 = 0\,V\ vs\ NHE$$

Apart from water oxidation and glycerol partial oxidation to produce C_3 products, there are other reaction routes to produce C_1 or C_2 products through C-C bond cleavage. The CO_2 is produced as a result of C-C bond dissociation at the anode. The product can be evaluated by

investigating the oxidation number compared to that of glycerol (carbon oxidation number of glycerol is -2, for acetol -2, lactic acid 0, CO_2 +4, DHA 0, formic acid 2, and acetic acid 0). For instance, acid-catalyzed dehydration leads to produce acetol as a non-oxidation product due to no net change in the carbon oxidation number compared to that of glycerol. For lactic acid formation, glycerol is oxidized to glyceraldehyde and then isomerized to lactic acid [8].

The complete oxidation (electrocombustion) of glycerol on the anode in acidic media can be stated by the following overall reaction [28]:

Equation 5-19 $$C_3H_8O_3 + 3H_2O \rightarrow 3CO_2 + 14H^+ + 14e^-$$

which occurred by the following mechanism. First, the adsorption of glycerol by the following reactions:

Equation 5-20 $$C_3H_8O_3 \rightarrow 3\,(CO) + 8H^+ + 8e^-$$

Equation 5-21 $$C_3H_8O_3 \rightarrow 3\,(COH) + 5H^+ + 5e^-$$

and then the adsorbed species get oxidized as follows:

Equation 5-22 $$(CO) + H_2O \rightarrow CO_2 + 2H^+ + 2e^-$$

Equation 5-23 $$(COH) + H_2O \rightarrow CO_2 + 3H^+ + 3e^-$$

High conversion rates are associated with glycerol decomposition into non-valuable products, such as CO_2 gas [7].

The selectivity of the products is highly dependent on the applied current, acidity and electrode type. According to the results in acidic media, higher anode potentials enhance C–C bond breakage of glycerol which is in accordance with Lee et al 2016 as well [25].

Formic acid may lead to the formation of CO_2. However, formic acid can also be dehydrated on Pt to produce carbon monoxide during glycerol oxidation [14]. Another possible reaction is producing CO is the direct oxidation of glycerol as follows:

Equation 5-24 $$C_3H_8O_3 \rightarrow 3CO + 4H_2$$

For acetic acid production the following reactions take place [22]:

Equation 5-25 $$C_3H_8O_3 \rightarrow \frac{3}{2}CH_3COOH + 2\,H_2$$

Equation 5-26 $$C_3H_8O_3 \rightarrow CH_3CH_2COOH + H_2O$$

Also, for formic acid production, several consecutive reactions occur including glyceraldehyde formation as follow [29]:

Equation 5-27 $$C_3H_8O_3 \rightarrow C_3H_6O_3 + H_2$$

Equation 5-28 $$C_3H_6O_3 + O_2 \rightarrow HCOOH + C_2H_4O_3$$

Equation 5-29 $$C_2H_4O_3 + \frac{1}{2}O_2 \rightarrow C_2H_2O_4 + H_2$$

Equation 5-30 $$C_2H_2O_4 \rightarrow 2CO_2 + H_2$$

Equation 5-31 $$CO_2 + H_2 \rightarrow HCOOH$$

The chlorine of HCl can also affect the reaction pathways by producing HClO and then oxidizing the glycerol. At the anode the following reactions are occurred [30]:

Equation 5-32 $$2Cl^- \rightarrow Cl_{2(aq)} + 2e^-$$

Equation 5-33 $$Cl_{2(aq)} + H_2O \leftrightarrow Cl^- + HClO$$

In acidic media, active chlorine presents as hypochlorous acid (HClO) (while the hypochlorite anion ClO^- would exist at higher pH [31]). Besides, formations of chlorate ClO_3^- and perchlorate ClO_4^- are not favourite on Ti/IrO_2 and Pt electrodes. Also, the production of gaseous chlorine Cl_2 from the solution at high temperatures is favoured.

Generally, Ti/IrO_2, Pt/Ti, and Pt electrodes have low values of oxygen evolution reaction (OER) overpotential and this point out that anodes with low OER overpotential values lead to the partial oxidation of organic matters (oxidation potential between 1.5-1.9 V and overpotential between 0.25-0.3). For the electrodes with higher OER overpotential, such as SnO_2, PbO_2 and BDD (boron doped diamond), the complete oxidation of organics (combustion) to carbon dioxide is preferred (oxidation potential in a range of 1.8-2.6 V and overpotential between 0.5-1.3). It is presumed that the initial reaction in both anode types (as M) links to the oxidation of a molecule of water results in the formation of hydroxyl radical (M(˙OH)) as the following reaction:

Equation 5-34 $$M + H_2O \rightarrow M(^{\circ}OH) + H^+ + e^-$$

The further reactions of heterogeneous M(•OH) (chemical or electrochemical) depend on the essence of electrodes. Actually, the surface of active electrode interacts intensely with •OH and the superoxide (MO) will be formed as follows (if the higher oxidation states are accessible beyond the standard potential for oxygen evolution for a metal oxide anode):

Equation 5-35 $$M(^{\circ}OH) \rightarrow MO + H^{+} + e^{-}$$

The redox couple MO/M is a mediator in the oxidation reaction (in competition with the OER), through the chemical decomposition of the superoxide species [32]:

Equation 5-36 $$MO + R \rightarrow M + RO$$

Equation 5-37 $$MO \rightarrow M + \frac{1}{2}O_2$$

Fig 5-7 shows the reaction pathway which involves the formation of C_1 to C_3. According to the density functional theory (DFT) calculations previously described by the researchers, the activation energy of C-C bond detachment is higher than that of C-H bond cleavage for glycerol and for adsorbed species on the electrode $(CHxOyHz)_{3,ads}$ throughout the primary step of dehydrogenation. Subsequently, the removal of hydrogen atoms from the intermediates occurs, and then the activation energies for both C-H and C-C bond dissociations turn into comparable, thus, on the electrode surface, C-H bond detachment takes place quickly, and afterward C-C bond dissociation proceeds to form the other adsorbed intermediates such as $(CHxOyHz)_{2,ads}$ and $CHxOyHz_{,ads}$. The oxidation of $CHxOyHz_{,ads}$, goes to form CO_2 and acetic acid (as C_2 product). Also, more complicated reaction chains are occurred to produce formic acid (oxidation followed by C-C bond dissociation). Moreover, adsorbed C_3 species will react via dehydration, and dehydrogenation to transform to other C_3 sorts, followed by desorption to form C_3 products like hydroxyacetone (acetol) by dehydration, DHA by dehydrogenation or glycidol by both dehydrogenation and dehydration [22]. Nevertheless, too high current density (1 A) could not assist a higher conversion of the glycerol to the added-value compounds, seemingly because it then facilitates the decomposition of glycerol to gaseous compounds, such as CO_2. Also, it was

observed that at higher current densities, the reaction goes to the reduction route to produce lactic acid [7].

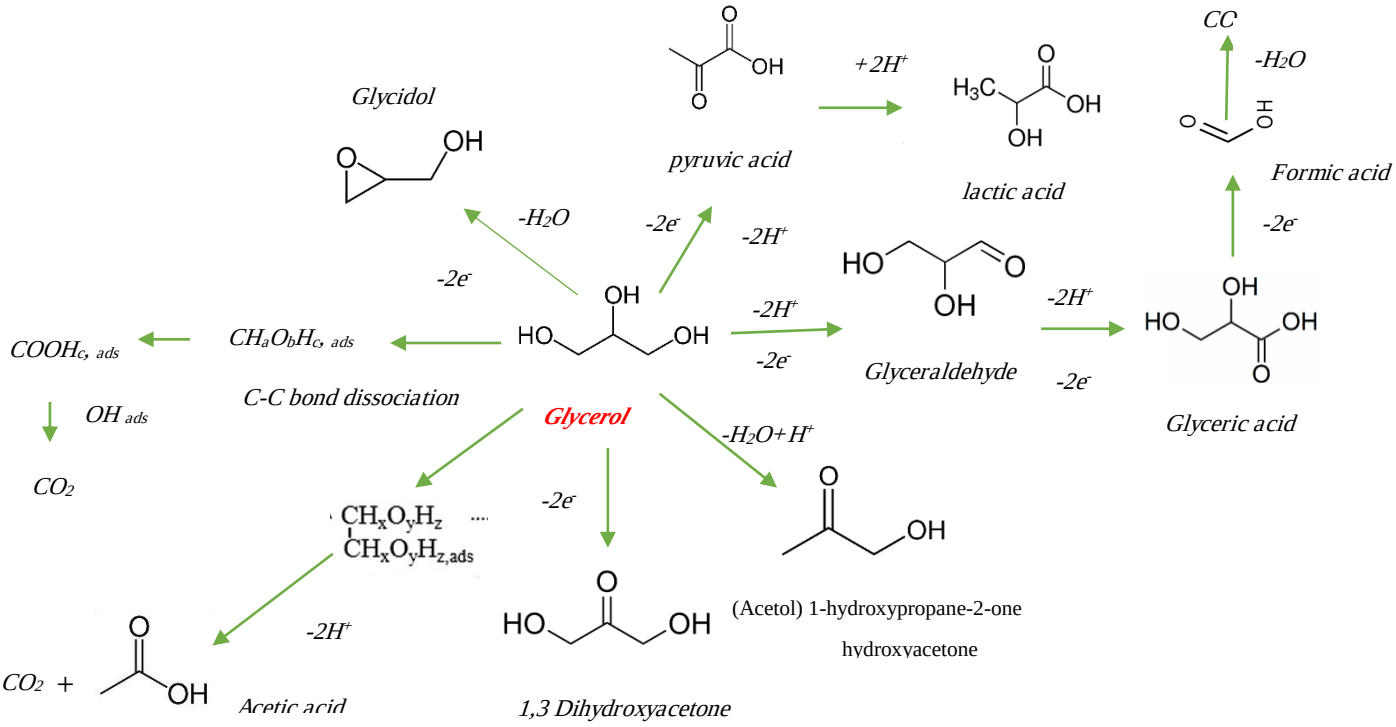


Figure 5-7 Reaction pathway of glycerol electrocatalytic conversion in this study.

5.4.6 Optimization

Optimization was performed using treated crude glycerol. The range of operating conditions was determined according to preliminary experiments and literature. Evaluating the 20 experimental data by the quadratic model was significant. The operating condition and the results are shown in Table 5-2. Mathematical quadratic model in terms of coded values (eq 5-38) is as follows:

Equation 5-38

$$S = 15084.27 + 861.95C + 2281.05\mathrm{A} + +2494.72\mathrm{G} + -65.50\mathrm{AC} + 213.75\mathrm{GC} + 78.25\mathrm{AG} - 6781.81C^2 - 3291.24A^2 - 2421.66G^2$$

where S is the sum of the concentrations (mg/L) of acetol, DHA, and glycidol, G is glycerol concentration (mg/L), C is current intensity (A), and A is HCl concentration. The coefficients of the model were computed using the half-difference between the arithmetic average of the response values when the associated coded variable is at a level (+1) and the arithmetic average of the response values when the associated coded variable is at level (−1). The surface 3D plots of the combined parameters of acid concentration, initial glycerol concentration, and applied current for maximum production of non-acidic compounds are shown in Fig 5-8 a/b/c. The plots clearly indicate that an optimum exists within the observed design space with respect to three mentioned parameters appears to increase solvent yield over the observed design space. Moreover, Table 5-2 presents the yield and selectivity at optimum condition. From this table, the maximum yield percentage of 72% of acetol could be achieved.

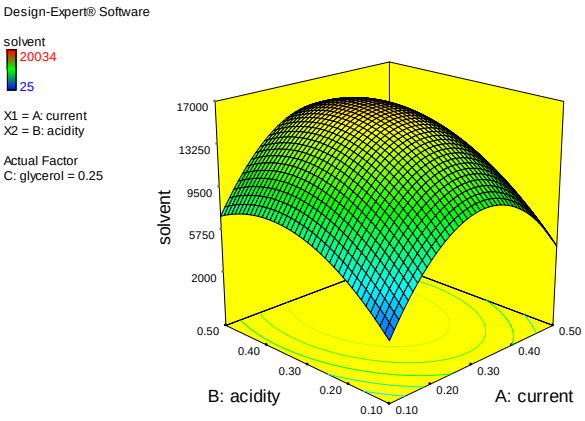
Moreover, in the range of selected operating parameters, the linear equation for acid productions was obtained as follows :

Equation 5-39

$$OA = 1123.08 + 749.09C + 4.8A + 190.52G$$

Where OA is organic acid concentration (mg/L). Also, the linear dependency of the acidic components production is shown in Fig E-5.

a



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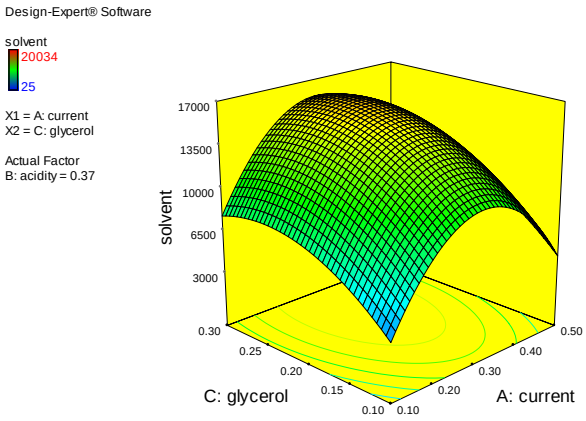

c

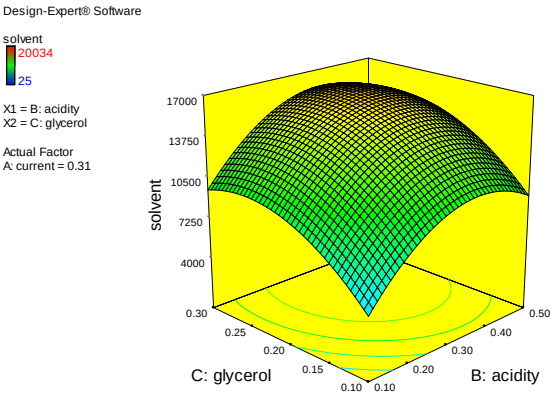

Figure 5-8. Optimization of acetol, DHA and glycidol production in terms of operating conditions

Table 5-2 **Optimum conditions for non-acidic production**

	Current (A)	Acid HCl (M)	Glycerol (M)	Non-acidic production (mg/L)			Acidic production (mg/L)		
Predicted	0.31	0.37	0.25	16170			1278		
Experimental	0.31	0.37	0.25	17201±9			1156±10		
				Acetol	DHA	Glycidol	Acetate	Lactate	Formate
				15021	1255	192	454	134	145
Yield[1] %				72.4	5.8	0.9	2.1	0.7	0.7
Selectivity[2]%				88.2	7.3	1.1	2.6	0.8	0.8

1 mass of product over the total mass of glycerol consumption
2 mass of specific product over the sum of all products

5.4.7 Overview of economic evaluation

The evaluation of operational costs was done by considering the electricity, chemicals, and glycerol consumptions. The energy consumed is estimated at a cost of 0.12 $/kWh. Chemical costs include crude glycerol. The total cost is evaluated in terms of US dollars spent per kg of CG (US$/kg). While the prices of glycerol oxidation products are moderately high, the market price of purified glycerol is relatively low [33]. crude glycerol is of little economic value, i.e., approximately $0.1/kg [34], The total benefits also including separation costs of the products which are not the subject of this article. This evaluation just aims to show the values of products from the non-expensive crude material. The price of purified glycerol worldwide is around USD $1.10/kg to $3.30/kg, which is 10 or more times higher than that of crude glycerol (USD $0.04/kg to $0.33/kg) [7]. Table 5-3 shows the simple calculation of total benefits by doing mass

and energy balance base on optimized conditions in this study. We also note that the operating costs especially the separation and purification of the products are very significant factors in economic evaluation, but it is not in our view in this study and can be performed later.

Table 5-3 **Economic evaluation (overview)**

	Energy kWh	**Crude Glycerol**	**Formic acid**	**Acetic acid**	**DHA**	**Acetol**	**Glycidol**	**Lactic acid**
Unit price $US/kg [7, 35, 36]	0.12 $/kWh	0.1	157.0	155.0	396.0	218.3	320.3	132.0
Energy consumption (kWh/kg glycerol)	0.31 kWh	1 kg	5g	16 g	44 g	535 g	68 g	5 g
Consumption/ production kg	-1.03 $	-28	0.14	0.45	1.25	15	1.92	0.13
Total cost $	-1.03 $	-2.8	+20	+70	+500	+3270	+615	+20

*The separation of products cost around 60-70% of total operating cost.

5.5 Conclusion

Electrocatalytic conversion of glycerol was performed in 500 ml batch reactor. An initial experiment using 10 g/L and 30 g/L of pure glycerol showed that the Pt electrode has the potential to produce value-added chemicals. In this research, the non-acidic product synthesis was investigated and optimized (using CCD method). Overall production throughout 12 h of operation was considered as optimization criterion; also, three variables were chosen as

glycerol concentration, acidity, and poised current using. This method can be used to remediate waste and produce value-added products simultaneously.

High oxidation of glycerol was observed at higher applied current but by increasing the current intensity the tendency of glycerol conversion to carbon dioxide and acidic products is increased. The electrochemical conversion of treated crude glycerol to various added-value compounds, such as glycidol, acetol, and DHA, was achieved under robust acidic condition (pH=1.4). Last but not least, comprehensive techno-economic evaluation should be performed to assess the feasibility of the process as this operation is at the early stage of development. Also, separation of products and recovery of acid from the solution must be investigated in future.

Acknowledgements

This work was supported by Quebec Research Funds Nature and Technologies (FRQNT).

Symbols and abbreviations

A	acid HCl concentration
Ag/AgCl	silver chloride electrode
ANOVA	analysis of variance
BDD	boron-doped diamond
b_0	average value of the responses of the assays
b_i	principal effect of each factor *i* on the response
b_{ij}	interaction effect between factor i and factor j on the response
C	current intensity
CCD	central composite design

CG	crude glycerol
DFT	density functional theory
DHA	dihydroxyacetone
G	glycerol concentration (g/L),
GC-MS	gas chromatography-mass spectrometry
HPLC	high pressure liquid chromatography
LC-MS	liquid chromatography-mass spectrometry
OER	oxygen evolution reaction
OA	organic acids
Pt	platinum
PtB	black platinum (carbon/platinum)
RSM	response surface methodology
SHE	standard hydrogen electrode
RHE	reversible hydrogen electrode
SS	stainless steel
S	solvents (acetol, DHA, glycidol)
T	temperature (^{o}C)
Ti/IrO_2,	titanium iridium oxide
TN	total nitrogen
TOC	total organic carbon
TSS	total suspended solids
X_i	coded variable
V	applied voltage (V)
Y	experimental response

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Annexe A: Article présenté dans une conférence

- **Ali Khosravanipour Mostafazadeh**, Patrick Drogui, Satinder Kaur Brar, Rajeshwar Dayal Tyagi, Yann Le Bihan, Gerardo Bueln, Electrochemical conversion of pure and crude glycerol to produce platform molecules, *The 2nd International Conference on Advanced Materials and Processes for Environment, Energy and Health, Montreal, Canada, Oct. 31st -Nov. 2nd, 2018.*

ABSTRACT

Glycerol is produced at 10 wt % of the total biodiesel production. The global production of bioglycerol is more than 2 million tonnes presently [1]. Nonetheless, the crude glycerol gained from biodiesel production has a low economic value. Different chemicals can be produced by oxidation, reduction, or dehydration of glycerol such as organic acids, solvents, and base chemicals. The product distribution highly depends on the geometric and electronic properties of the electrodes, the glycerol concentration, and the operating conditions of the process, such as electrolyte solution, pH, temperature, etc. [2].

Production of platform molecules such as acetol, acrolein, acetic acid, propionic acid, etc. from pure glycerol and pretreated crude glycerol solutions in water (0.1-0.3 M) using rectangular platinum, and Pt/Ti anodes, and Pt/Ti, or stainless steel cathodes by applying current density between 1 to 20 mA in a bench scale electrochemical cell (450 ml) are performed. Moreover, investigation of the effects of pH (acidic, neutral or basic), and electrolyte were investigated. The results showed that the product distributions highly depends on electrode material (as a catalyst) and the pH of the solution. Figure A. 1. Shows the overall reaction pathway of glycerol conversion into value added products.

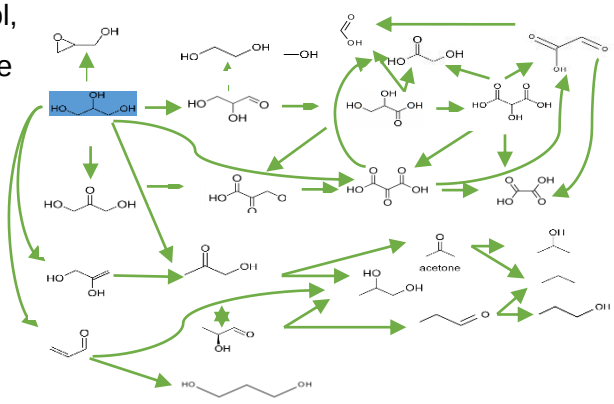

Figure. A. 1. General mechanism of electrocatalytic conversion of glycerol

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Annexe B: Données complémentaires au chapitre 3

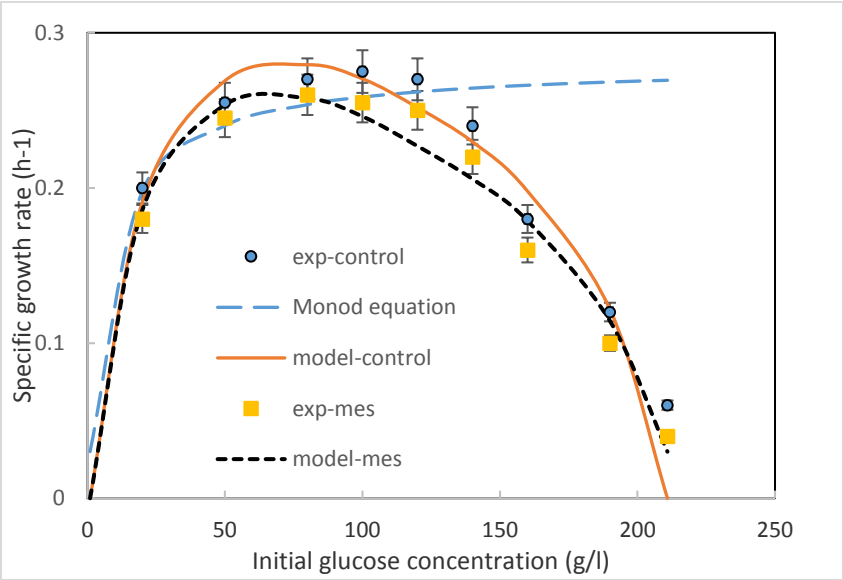


Figure B.1. The specific growth rate of *C. pasteurianum* at different substrate concentrations (experimental and model results).

Annexe C: Méthodes analytiques

La figure c.1 montre les pics d'analyse des produits de l'électrosynthèse microbienne tels que le butanol et le propanediol par GC.

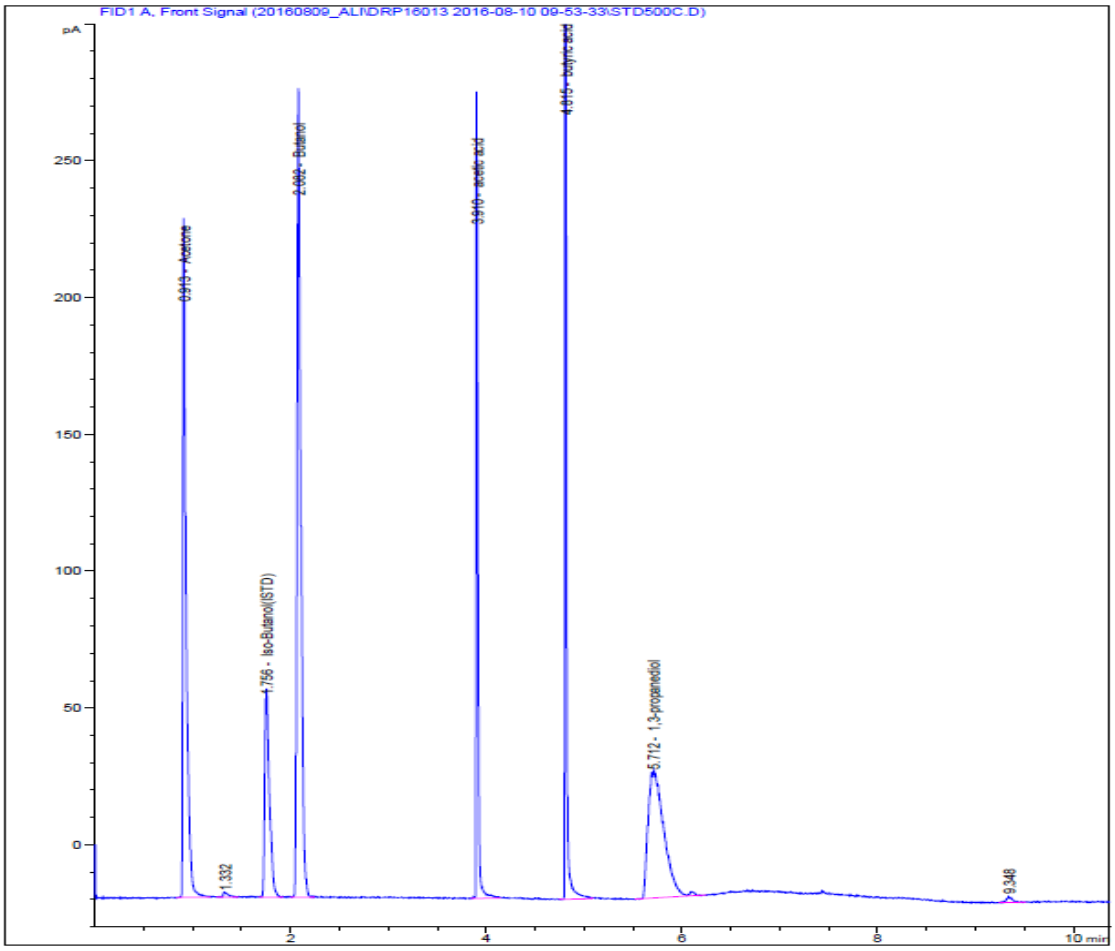


Figure C.1. Pics de chromatographie en phase gazeuse pour l'analyse de l'électrosynthèse microbienne.

C.1. Analyse du Glycerol

Méthode LC: *glycerol20180226.meth;* Process method: *glycerol_calc*

Conditions chromatographiques (HPLC)

Système : Finnigan Surveyor LC Pump Plus et Autosampler Plus

Colonne: Hypersil Gold Amino 150 x 2.1mm x 3µm

Oven: 35 ^{o}C

Débit : 0.25 mL/minute

Volume d'injection : 10 µl

Température du Tray: 25 ^{o}C

Phase mobile : A: H2O 0.1% NH_4OH
D: ACN 0.1% NH_4OH

Gradient: isocratique A:15% D:85% pendant 11 minutes

Pression attendue : ~2200 psi

Conditions du ESI et du spectromètre de masse

Système : TSQ Quantum Access Mass Spectrometer

Interface: Electrospray Ionization Source (ESI)

Mode d'ionisation : Négatif

Position de la probe : ``D``

Spray voltage: 4000 V

Sheath gas: 45 arbitrary units

Auxiliary gas: 25 arbitrary units

Ion sweep gas: 0 arbitrary units

Capillary température : 325 °C

Collision gas : Argon à 1.1 mTorr

Skimmer offset: 3 arbitrary units

Operating mode: Selected Reaction Monitoring (SRM)

Tableua C.1. Préparation de la courbe

Std #	[*] (ppm)	Vol. ACN : H_2O (85:15) (µL)	Vol Std Ajout (µL)	Std Ajout
1	5	995	5	1000 ppm
2	10	990	10	1000 ppm
3	20	980	20	1000 ppm
4	50	950	50	1000 ppm
5	100	900	100	1000 ppm

Volume de ISTD = 20µL glycerol- d_8 (500 ppm) pour 1000µL total.

Pour les dilutions des échantillons : S'il est nécessaire de faire deux dilutions (ou plus) pour les échantillons, la première dilution doit être faite dans l'eau, et la dernière dans ACN :H_2O 85 :15

Stockage de la colonne: Faire pomper pendant 30 minutes de l'éthanol 95%

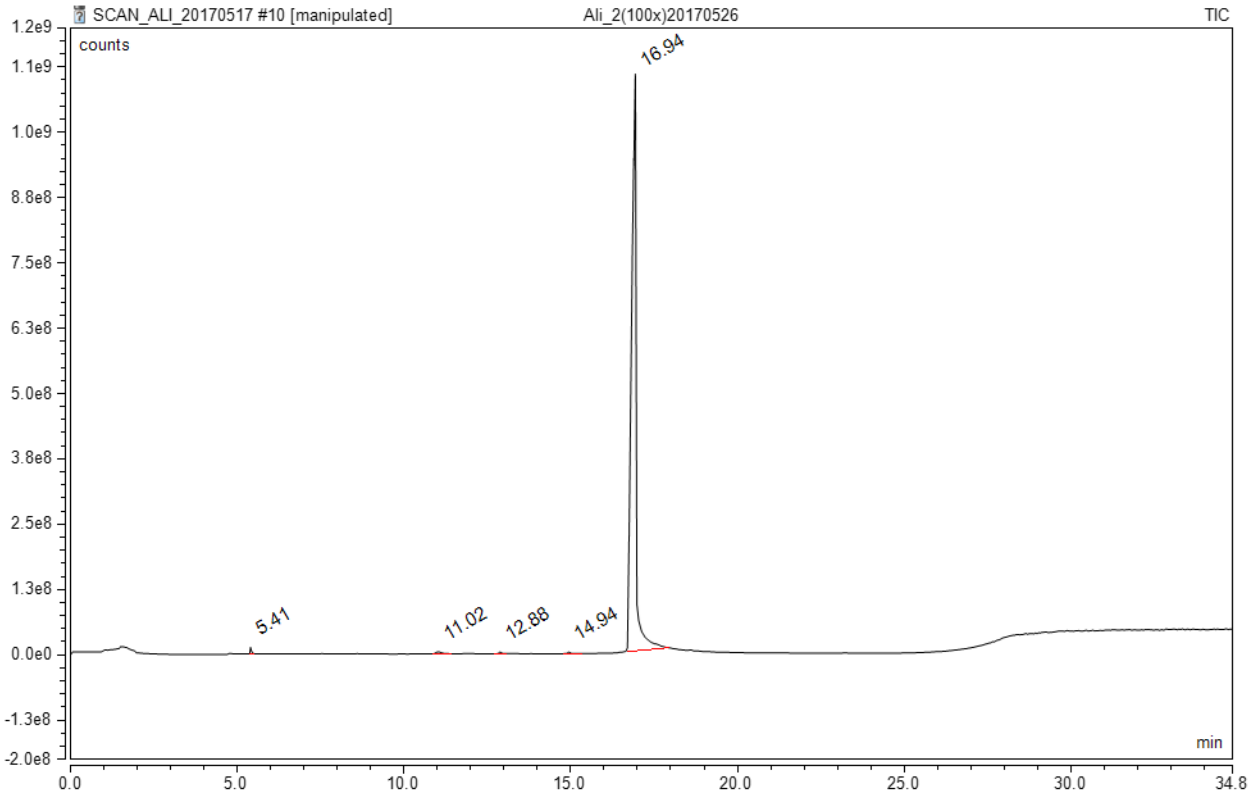


Figure C.2. Crude glycerol analysis by GC/MS: R.Time %, compound, 16.94,921 Glycerin

C.2. Instrument parameter for glycerol electrochemical conversion (Scan)

Instrument: Thermo Scientific GCMS model ISQ

Column: DB-WAX 30m x 0.2mm i.d. x 0.2um film thickness

Instrument Setup:

GC.Front Inlet Mode: Splitless

GC.Front Inlet.Temperature: 225 [°C]

Column flow: 1.0 ml/min (carrier gas: Helium)

Oven program:

Initial Temperature:	60°C for 1 min
Ramped:	8°C/min until 200°C
Stay at:	200°C for 6 min
Ramped:	15°C/min until 250°C
Stay at:	250°C for 7 min
Run time:	34.8 min

MS detection mode: Full Scan (range 30-400 m/z)

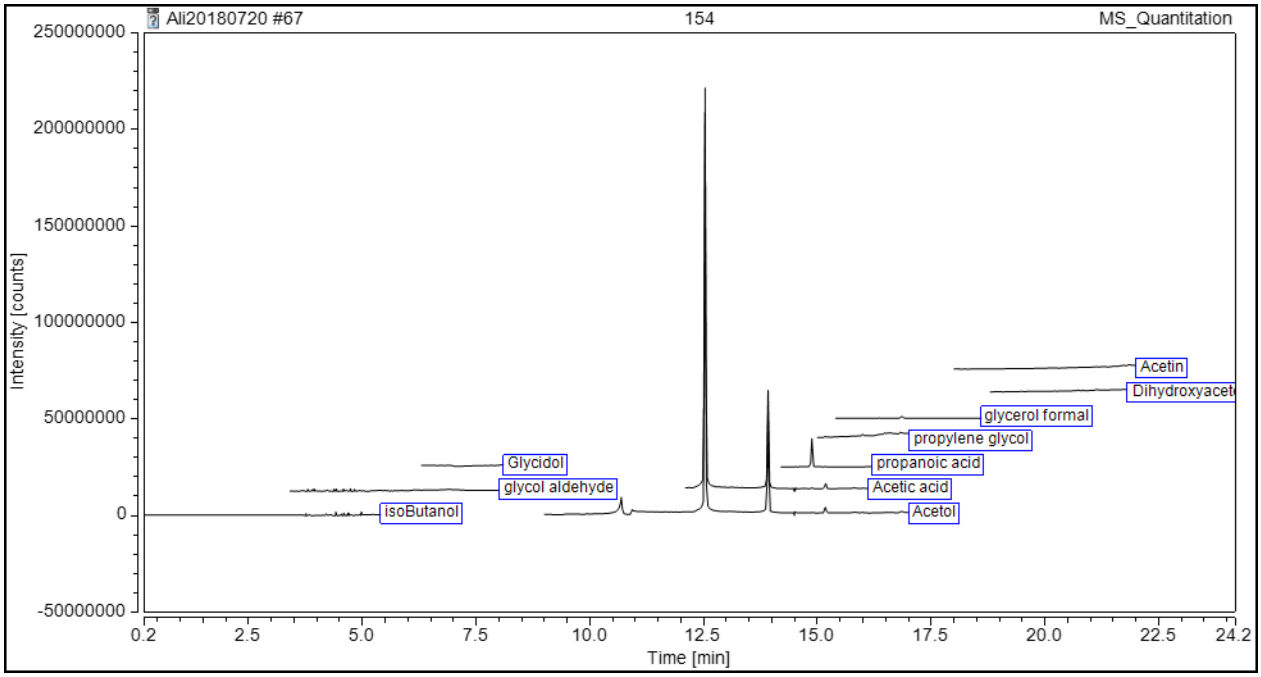
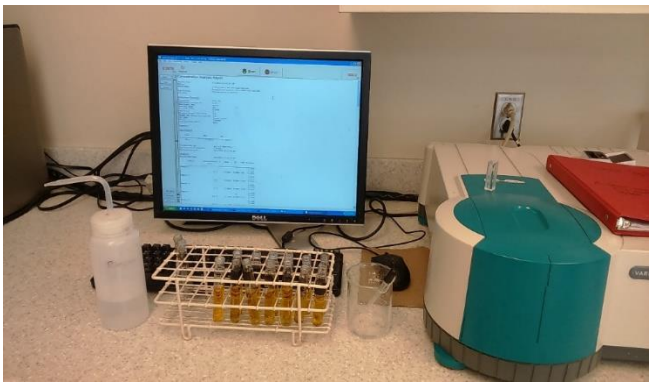


Figure C.3. Standard sample analysis for glycerol electrocatalytic conversion in GC/MS

a)



b)

Figure. C.4 a) Gas chromatograph, b) spectrophotometer UV/VIS

Annexe D: Données complémentaires au chapitre 4.

Table D.1. Glycerol 1,3 PD data for optimisation

Run	T (°C)	Glycerol (g/L)	Voltage (V)	1,3 PD (g/L)
1	33.50	35.00	0.00	4.7
2	37.00	20.00	2.00	4.5
3	37.00	50.00	0.00	4.4
4	39.39	35.00	1.00	5.9
5	33.50	9.77	1.00	3.5
6	37.00	20.00	0.00	3.8
7	30.00	50.00	2.00	6.3
8	37.00	50.00	2.00	6.8
9	33.50	35.00	1.00	6.95
10	27.61	35.00	1.00	5.1
11	30.00	50.00	0.00	4
12	30.00	20.00	0.00	3.3
13	33.50	60.23	1.00	5.7
14	33.50	35.00	2.68	6.7
15	33.50	35.00	1.00	6.97
16	33.50	35.00	1.00	6.95
17	30.00	20.00	2.00	4.1
18	33.50	35.00	1.00	6.97
19	33.50	35.00	1.00	6.95
20	33.50	35.00	1.00	7

Table D. 2. Overview of the economic evaluation of microbial electrosynthesis

For 10 kg of butanol or 1,3 PD	Glucose (MES)	Glucose (CF)	Crude glycerol (MES)	Crude glycerol (CF)
Substrate cost $	-40	-57	-3	-5.5
Medium	-8	-11.5	-8	-11.5
electricity	-7 cent		-7 cent	0
Butanol	860$	640$		
1,3 PD			1310 $	1310 $

MES: microbial electrosynthesis

CF : conventional fermentation

For 1 kg of glucose: energy consumption is 0.015 kWh

Annexe E: Données complémentaires au chapitre 5.

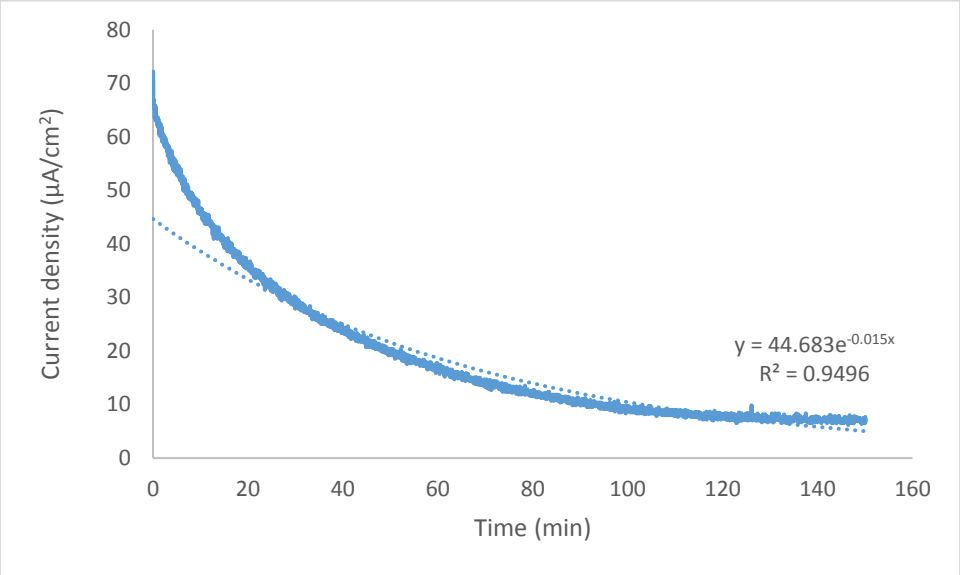
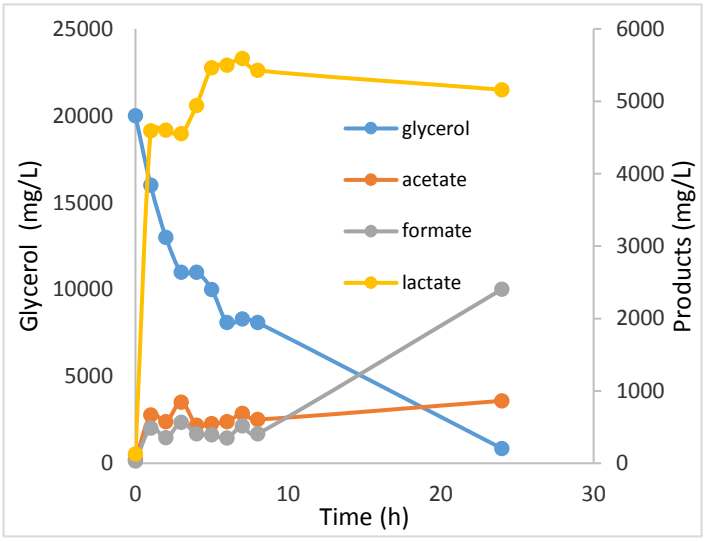


Figure E. 1. Decay curve of catalyst deactivation model



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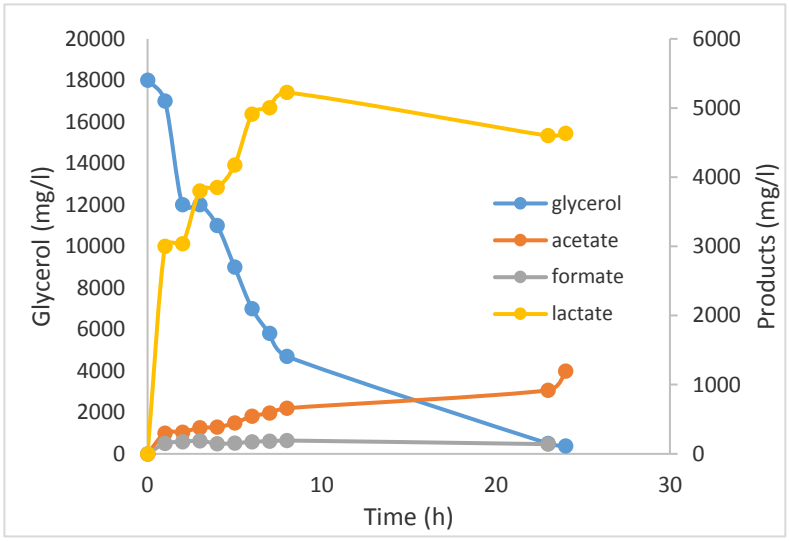
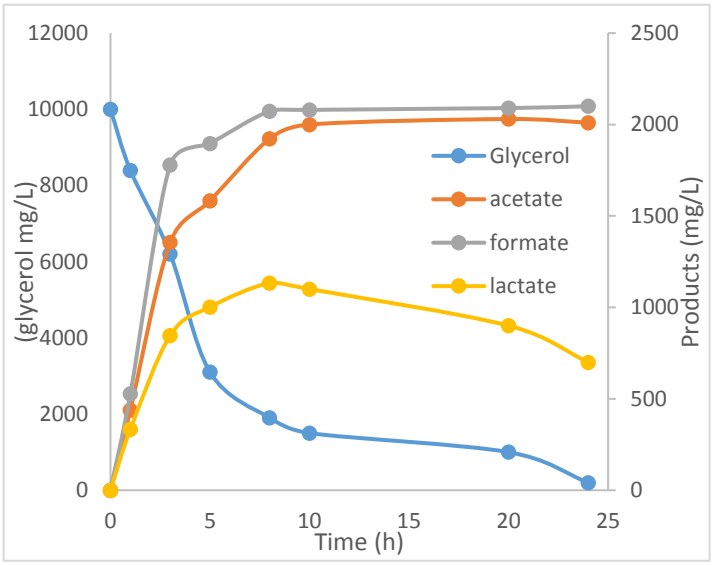


b



c

Figure E. 2. Glycerol conversion and products distribution under alkaline condition NaOH 0.5 M, a) I=0.5 A, Pt black as the anode, b)I= 1 A, Pt black as an anode, c) I=1 A, Pt as an anode.

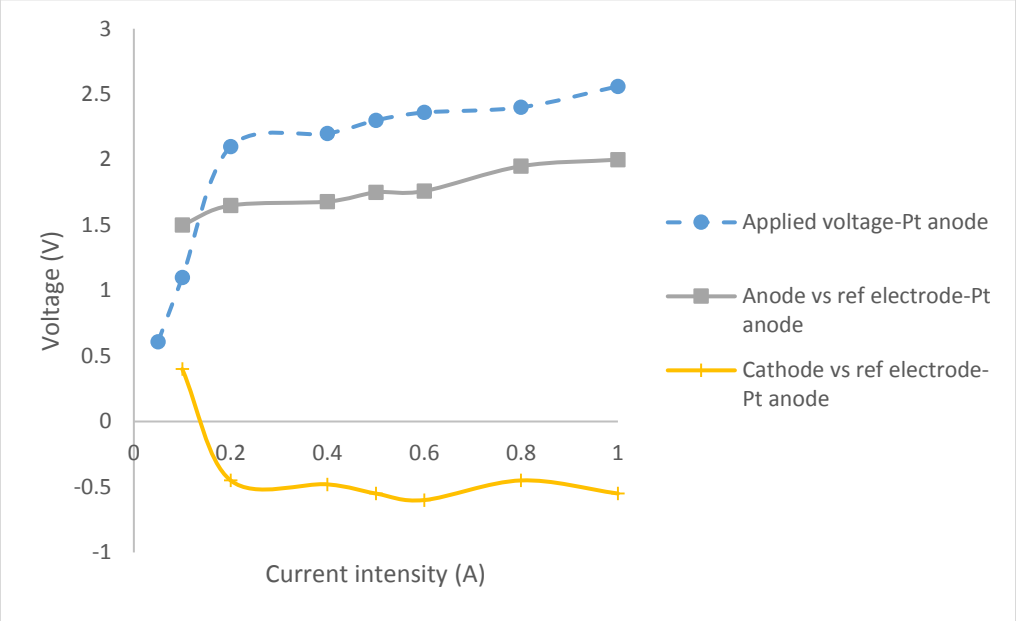
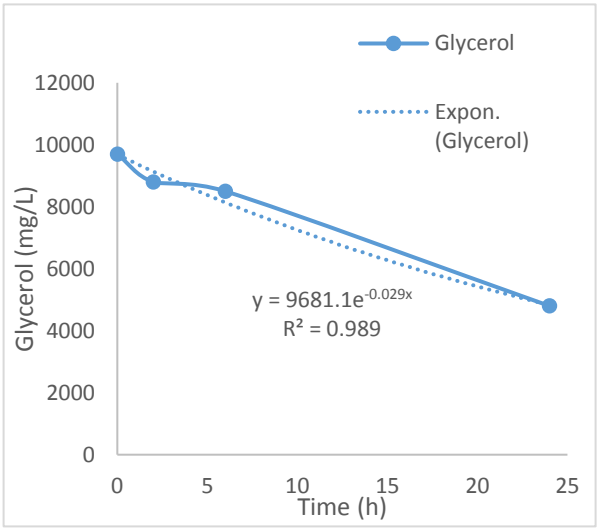
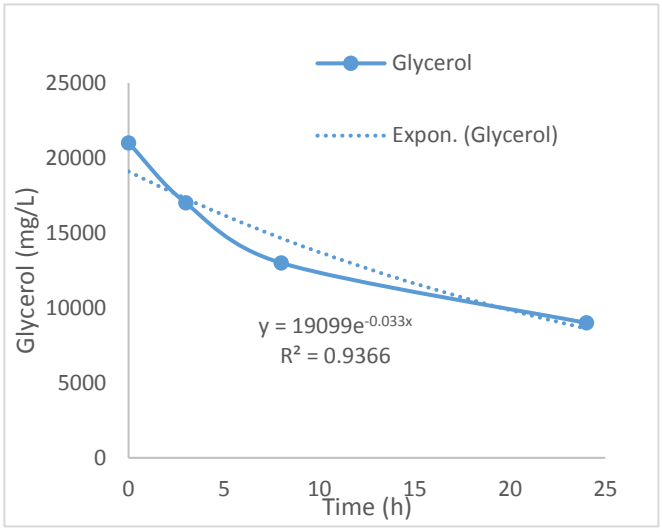


Figure E.3. Applied voltage and the voltage between the reference electrode (Ag/Agcl; 3 M) for the glycerol electrochemical conversion by using the Pt electrode.



a



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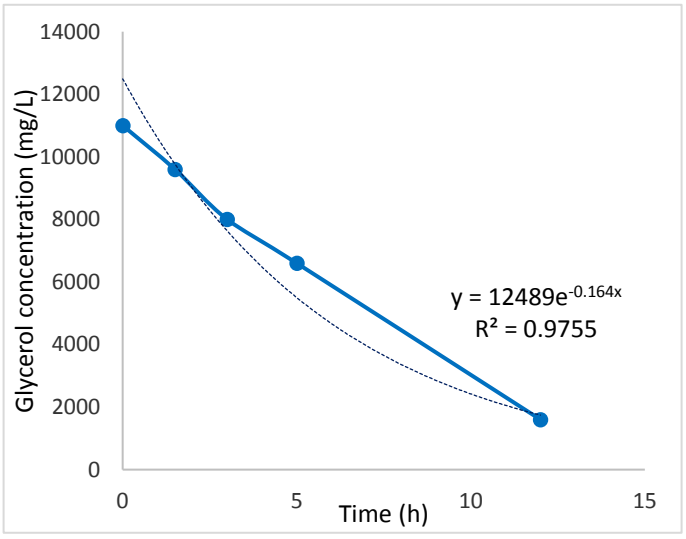
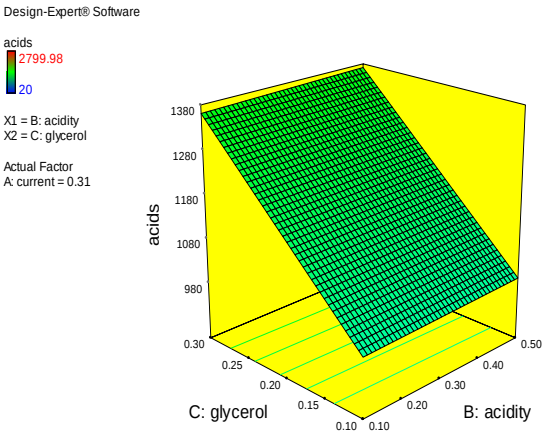


c

Figure E.4. kinetic models of glycerol conversion by using Pt electrode at acidic solution HCl 0.5 M; a) 0.3 A, b) 0.5 A, c) 1.0 A.

a



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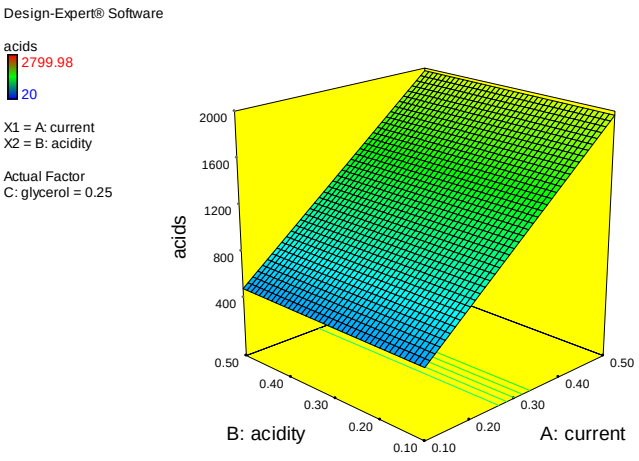


Figure E. 5. Organic acids production prediction in terms of operating conditions

Annexe F: Normalisation de la configuration du réacteur

The bioreactor is made from borosilicate glasses. Most characteristics of the glass reactor are attributable to metallic oxides, and the "boro" in borosilicate is a reference to boron. Borosilicate glass has been a primary choice for research and industry because of its low thermal expansion, high surface strength, and its high resistance to acids, salt solutions, and organic substances. The borosilicate glass reactor has a linear coefficient of expansion of 32.7 x 10^{-7} cm/cm/deg. C.

O-ring joints are connections made by two identical parts, each with an O-ring groove in the face, sealed with an O-ring, and held together by clamps. They do not need grease and can provide a good vacuum tight seal. O-rings are available in a variety of elastomers, Viton being the most common. Care must be taken to clamp adequately, and to avoid torque when connecting flexible tubing; heavy vacuum tubing can tweak even very strong clamps if it is asked to bend too sharply. Figure A shows the configuration of the reactor used in bench scale.

Figure F.1: Bioreactor set-up

This cell configuration is designed for research. The cells cover a lot of the basic needs for the area of investigation and are adaptable to other applications as well. Microbial electrosynthesis and electrochemical conversion in reactors are essentially modified in H-cells, to be used with various membranes sandwiched between the flanges. These flanges are called-out by their I.D. and the most common sizes are 25mm, 40mm, and 50 mm. The ports are glass threads that use sealing rings to hold electrodes, hose barbs for liquid flow, or Teflon-faced septa for needle sampling. These cells are constructed on standard borosilicate media bottles and fitted with robust threaded glass side ports. Typically, the reactors have 350 ml volumes with three different options for porting: hose barbs, Teflon faced silicone septa for piercing for sampling and sealing rings to hold electrodes. The thickness of the intended membrane has an effect on the clamping of the flanges; a membrane thicker than 0.015" will require some accommodations in order for the clamp to hold correctly.

Table F.1. Data used to perform the experiment in the reactor

Parameter	Applied voltage/ current density	Temperature	pH	Concentration	Size and type of electrodes	The working volume of the reactor	Operating time
Microbial electrolysis	0-3 V	30-37 ^{o}C	6.5-7	10-140 g/L depends on feed type	Carbon-based Stainless steel 3*10 cm	300 mL(anode) 300 mL (cathode)	Up to 72 h
Electrochemical conversion	0-0.5 A/cm^2	Ambient	acidic	0.1 to 0.3 M	Mesh Pt/Pt-Ti 11*10 cm	500 mL	Up to 24 h

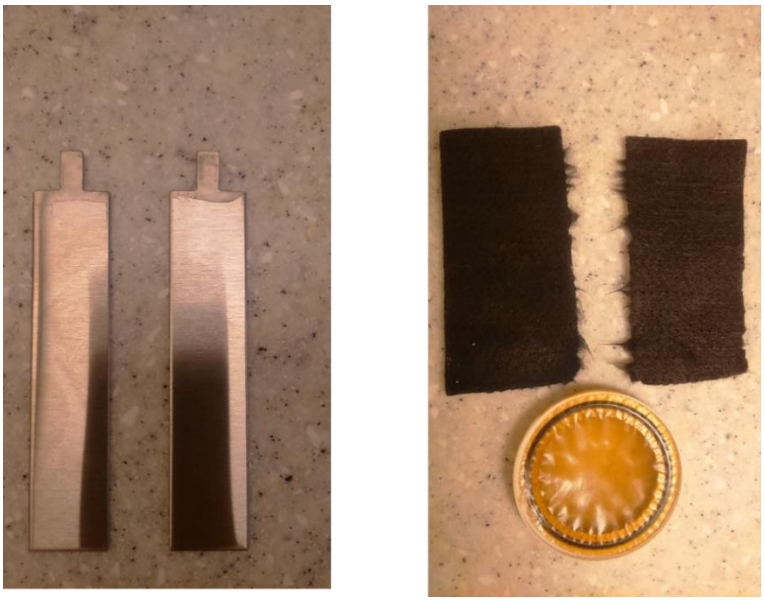


Figure F.2. Electrodes and membrane used in this study: left: SS electrode, right: graphite felt electrode with Nafion membrane

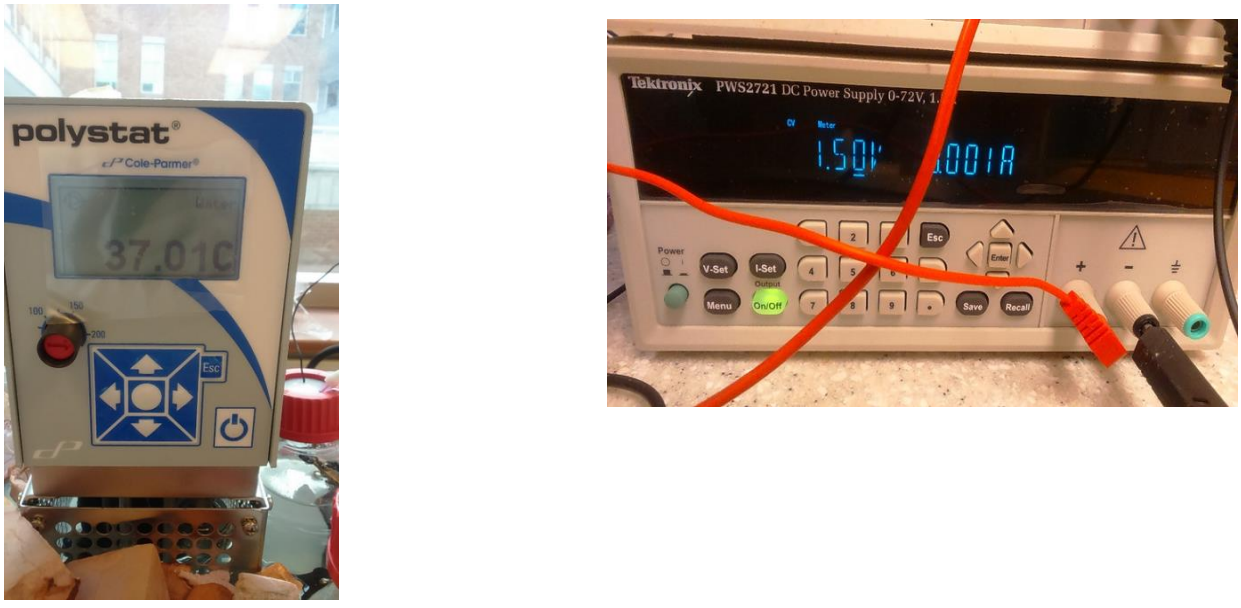
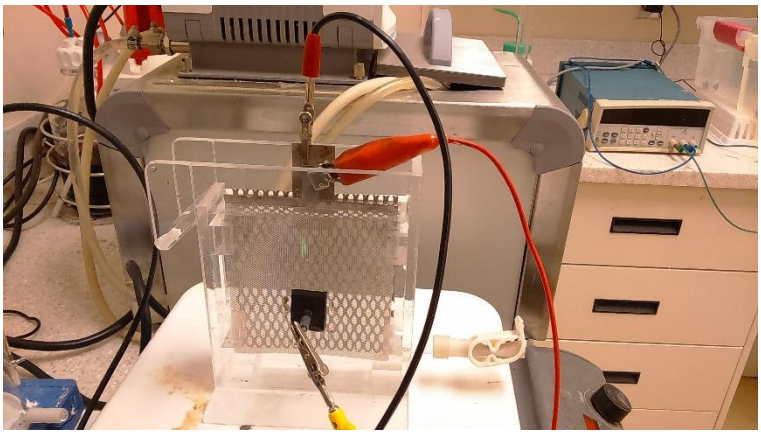


Figure F.3. left: Heater for temperature control, and right: electricity generator (constant voltage or constant current).



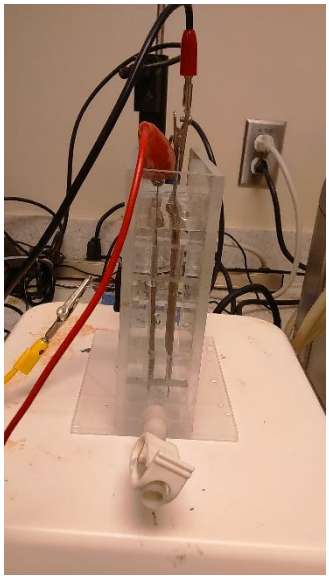


Figure. F.4. Electro-reactor for glycerol electrochemical conversion

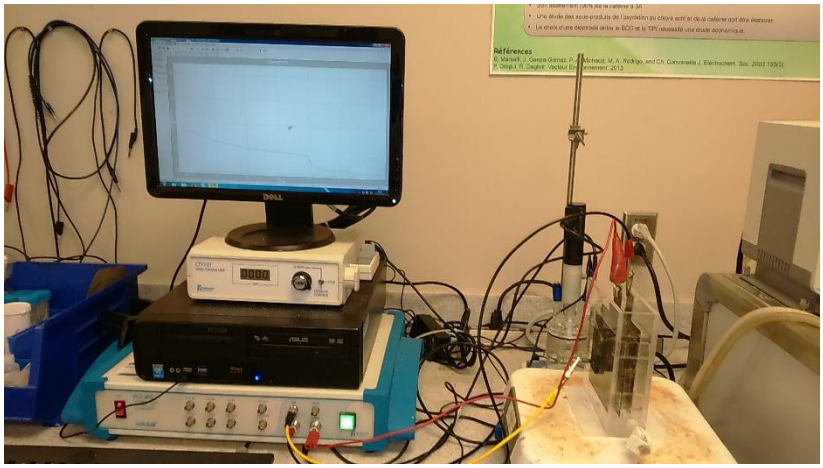


Figure F.5. Cyclic voltammetric study by the potentiostat