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LISTE DES ABRÉVIATIONS

RTA	Rio Tinto Alcan
UQTR	Université du Québec à Trois-Rivières
C	Carbone
CRIEB	Chaire de recherche industrielle en environnement et biotechnologie
CRIQ	Centre de recherche industrielle du Québec
Fe	Fer
g	Gramme
GG	Glucose - galactose
hPL	Poudre de lactosérum hydrolysée
INO	Institut national d'optique
j	Jour
L	Litre
Mg	Magnésium
N	Azote
nhPL	Poudre de lactosérum non hydrolysée
P	Phosphore
p	Poids
TAG	Triacylglycerol
v	Volume

CHAPITRE I

INTRODUCTION

1.1 Les microalgues comme source d'énergie

La fin de la dernière décennie fut marquée par le constat mondial d'un épuisement de plus en plus imminent des ressources fossiles, qui sont d'une importance capitale pour l'économie actuelle (Shafiee et Topal, 2009). Bien qu'il semble que la récente exploitation de réserves non-conventionnelles de gaz et de pétrole repousse ce délai, elles n'en sont pas moins limitées (Höök et Tang, 2013). En raison de cet éventuel épuisement des ressources fossiles, plusieurs industries cherchent à sécuriser leur approvisionnement énergétique pour le futur. Point de départ pour la recherche de sources renouvelables, cette motivation rejoint par le fait même les préoccupations environnementales actuelles. Ainsi, la culture intensive de microalgues à des fins énergétiques semble être une avenue prometteuse pour répondre à ces besoins grâce à leur productivité élevée en termes de biomasse et leur teneur en lipides (Mata, Martins *et al.*, 2010). D'autant plus que divers types de carburants peuvent être produits avec la biomasse algale : du combustible à partir de la biomasse sèche (Pittman, Dean *et al.*, 2011), de l'huile brute par liquéfaction hydrothermale (López Barreiro, Prins *et al.*, 2013), du biodiesel à partir de l'huile extraite (Demirbas et Fatih Demirbas, 2011), ou encore du biogaz par méthanisation de la biomasse résiduelle (Chisti, 2007; Singh et Gu, 2010; Pittman, Dean *et al.*, 2011) en sont des exemples.

1.2 La cohabitation : approche avantageuse pour la production de microalgues

Bien que ne procurant pas pour le moment une rentabilité concurrentielle aux ressources fossiles, la recherche très active dans le domaine des algocarburants contribue constamment à l'améliorer (Ziolkowska et Simon, 2014). Estimé à 6,00 $ en 2011 par la compagnie Oilgae (Oilgae, 2011), le coût de production d'un gallon (US) de diesel serait

maintenant passé à 2,28 $ selon les estimations de la compagnie Originoil (Ziolkowska et Simon, 2014). Cette dernière estimation concerne des productions de microalgues riches en huiles dans des eaux usées chargées en nutriments.

L'implantation de concepts intégrés de culture d'algues dans des industries existantes est une voie de développement favorisant leur rentabilité (McGinn, Dickinson *et al.*, 2011; Lohrey et Kochergin, 2012). Par exemple, le recyclage des eaux usées (Pittman, Dean *et al.*, 2011) et de certains résidus contenant des nutriments (Perez-Garcia, Escalante *et al.*, 2011) ainsi que l'utilisation de chaleur résiduelle émanant des procédés de production en usine (Xu, Brilman *et al.*, 2011) diminuent les coûts de production liés à la culture d'algues. Le gaz carbonique (CO_2) de leurs effluents gazeux peut aussi être utilisé par les algues comme source de carbone, ce qui contribue à la réduction des gaz à effet de serre émanant du système (Brennan et Owende, 2010; Hundt et Reddy, 2011; Rosenberg, Mathias *et al.*, 2011). D'autre part, le remplacement d'une partie des carburants et combustibles fossiles utilisés dans les procédés de production de l'industrie par un biocarburant, un biogaz ou une bioénergie à base de biomasse algale produite sur place diminue les coûts liés à la consommation de carburant. La cohabitation d'une production de microalgues avec une industrie offre ainsi plusieurs avantages.

1.3 Projet dans lequel s'intègre l'étude

Comme d'autres industries, la compagnie Rio Tinto Alcan (RTA) a entrepris d'étudier la possibilité d'utiliser de la biomasse pour remplacer une partie de l'énergie et des carburants fossiles utilisés en usine. Devant la possibilité de valoriser ses résidus ainsi que de réduire ses empreintes carbone et énergétique, la compagnie a choisi d'évaluer la faisabilité de produire de la biomasse algale. C'est ainsi que RTA, la Chaire de recherche industrielle en environnement et biotechnologie (CRIEB) de la Fondation de l'Université du Québec à Trois-Rivières (UQTR) et la compagnie Alga-Labs (Montréal, QC) ont lancé en mai 2011 un projet sur la culture intensive de microalgues à haute valeur calorifique intégré à une usine RTA au Québec. Ce projet, s'échelonnant

sur quatre ans, visait à développer un procédé de culture de microalgues dans les eaux usées de l'industrie en bassin extérieur adjacent à l'usine, sur une superficie minimale et viable économiquement. La cohabitation d'une production de microalgues et de l'usine RTA permettrait l'utilisation des eaux usées et de résidus comme milieu de culture, des effluents de CO_2 comme source de carbone, et de chaleur résiduelle émanant des procédés pour maintenir la température des bassins.

Plusieurs auteurs ont exposé les bénéfices que pouvait procurer la production de microalgues à des fins énergétiques dans des eaux usées autant en ce qui concerne la rentabilité que les bienfaits environnementaux (Chinnasamy, Bhatnagar *et al.*, 2010; Park, Craggs *et al.*, 2011; Pittman, Dean *et al.*, 2011; Abdelaziz, Leite *et al.*, 2013; Borowitzka et Moheimani, 2013). Bien que la concentration en carbone, azote et phosphore dans les eaux usées industrielles soit plus faible que dans celles provenant du secteur agricole, alimentaire ou du traitement municipal, diverses études ont démontré qu'elles pouvaient supporter la croissance d'algues. Par exemple, Chinnasamy, Bhatnagar *et al.* (2010), qui ont étudié en laboratoire un consortium d'algues composé de *Chlamydomonas globosa*, *Chlorella minutissima* et *Scenedesmus bijuga*, ont estimé qu'une productivité en biomasse de 17,8 tonnes ha^{-1} an^{-1} pourrait être atteinte dans des eaux usées d'industries de fabrication de tapis. Ces eaux usées contenaient des produits chimiques de procédés, des pigments et divers éléments inorganiques comme de l'azote, du phosphore et des métaux en faibles concentrations (Pittman, Dean *et al.*, 2011). La productivité obtenue pourrait, selon les auteurs, soutenir une production de biocarburant à base d'algues. De plus, une étude de Tarlan, Dilek *et al.* (2002) a révélé la capacité d'un consortium de plusieurs espèces d'algues à croître dans des eaux usées d'industries papetières et à réduire leur charge en nutriments. Une autre étude (Lim, Chu *et al.*, 2010) visant la phycoremédiation d'effluents issus d'une usine de textile a démontré le potentiel de la souche *Chlorella vulgaris* UMACC 001 pour la production de biomasse à des fins énergétiques dans ces eaux usées. Le taux de croissance spécifique rapporté dans le milieu composé d'eaux usées à 80 % (0,21 $\mu \cdot jour^{-1}$) était similaire à celui obtenu dans le milieu standard Bold Basal (0,28 $\mu \cdot jour^{-1}$). Wu, Chen *et al.* (2012) ont également démontré la faisabilité de produire de la biomasse avec la

souche *Chlamydomonas sp.* TAI-2 dans des eaux usées d'une composition complexe provenant d'un parc industriel. L'objectif était de produire du biodiesel tout en les épurant, par la diminution de la charge en azote et en phosphore. Dans ce même but, plusieurs études ont aussi été réalisées sur les eaux usées produites lors de l'extraction de gaz de schale (Hamawand, Yusaf *et al.*, 2014). Leur concentration élevée en sodium pourrait être réduite par un enrichissement en acide acétique, qui réagirait avec le sodium pour former de l'acétate de sodium, subséquemment consommé comme source de carbone par les microalgues.

Très peu d'études ont toutefois été publiées sur l'utilisation des eaux usées d'aluminerie pour produire des microalgues. Les recherches concernant celles-ci portent principalement sur la toxicologie. Ces eaux usées contiennent de l'aluminium et du fluorure qui peuvent avoir un effet néfaste sur la croissance des microalgues, mais qui peuvent également représenter un avantage pour la culture sous des conditions non aseptiques, si les souches sont robustes et résistent à ces facteurs toxiques. Une étude de Rai, Husaini *et al.* (1998) s'est penchée sur l'effet des composés à base de fluor et d'aluminium sur une espèce de *Chlorella*. Les résultats démontrent que l'algue peut absorber ou adsorber ces composés et que la dynamique des échanges dépend de la concentration de chacun ainsi que des phosphates. La prise de nutriments et la photosynthèse pouvaient toutefois être perturbées par la présence d'aluminium et de fluorure.

Néanmoins, soulignons l'étude en cours du Centre de recherche industrielle du Québec (CRIQ), en collaboration avec l'Institut national d'optique (INO) et Aluminerie Alouette. Elle porte sur le développement d'un système « maximisant le processus de photosynthèse à l'aide d'un système de captage et de redistribution de la lumière naturelle dans un bassin d'une profondeur supérieure à 30 cm » (CRIQ, 2014). Cette étude s'insère dans un programme de recherche axé sur la bioséquestration et valorisation énergétique du dioxyde de carbone par les microalgues dans un contexte climatique canadien. Financé par différents ministères et organismes, il découle de l'Action 20 du Plan d'action québécois sur les changements climatiques 2006-2012

(Économie, Innovation et Exportation Québec, 2011). Cependant, cette étude porte sur les gaz et non sur les effluents d'une aluminerie.

Le système de culture envisagé pour la production de biomasse algale dans le projet de RTA est le bassin Alga Fuel^{TM}, conçu par la compagnie Alga-Labs, qui requière un mode de croissance des microalgues de type mixotrophique (Dubois-Calero et Magnin, 2011). Ce mode trophique implique un apport en CO_2 et en carbone organique en présence de lumière puisqu'il fait intervenir à la fois le mécanisme de la photosynthèse pour la production de sucres endogènes et la consommation de sucres exogènes (Perez-Garcia, Escalante *et al.*, 2011). En outre, plusieurs études sur *Chlorella sp.* et d'autres espèces démontrent que la production de biomasse et de lipides en mixotrophie est supérieure à celle produite en photoautotrophie (Liang, Sarkany *et al.*, 2009; Heredia-Arroyo, Wei *et al.*, 2010; Wan, Liu *et al.*, 2011).

Deux souches de microalgues ont jusqu'à maintenant été étudiées dans le projet. Il s'agit de consortiums algues-bactéries isolés à même les eaux usées de RTA par la compagnie Alga-Labs, et composés majoritairement de *Chlorella sp.* Une étude de Bhatnagar, Chinnasamy *et al.* (2011) mentionne d'ailleurs que l'utilisation d'un consortium d'algues rendrait le système en bassin ouvert plus stable et robuste, d'autant plus si la production est faite dans des eaux usées dont la composition est variable. Safonova, Kvitko *et al.* (2004), qui ont plutôt étudié un consortium algues-bactéries, arrivent à la même conclusion. La spécificité et la variabilité des contaminants résiduels des eaux usées de l'aluminerie à laquelle la souche utilisée est adaptée favorisent également sa dominance sur les potentiels contaminants biologiques tels que bactéries indésirables, champignons, protozoaires et rotifères. Certaines études soulignent que la sélection d'une souche d'algues capable de croître dans un environnement non favorable procure un avantage compétitif sur les autres organismes indésirables (Lee 2001, Koller, Salerno *et al.*, 2012). Donc, malgré la culture mixotrophique en conditions non aseptiques, la robustesse et la stabilité du consortium utilisé devront permettre d'atteindre une productivité adéquate. Perez-Garcia, Escalante *et al.* (2011) soulignent d'ailleurs que la première qualité d'une souche est sa robustesse, c'est-à-dire sa stabilité

face aux fluctuations des conditions environnementales, y compris la compétition. Cette stabilité est également soutenue par la technique de culture développée par Alga-Labs impliquant entre autres l'utilisation d'un inoculum concentré et un apport en carbone organique ponctuel. Selon une étude de Zheng, Chi *et al.* (2012) l'utilisation d'un inoculum concentré pourrait effectivement limiter la contamination par des espèces d'algues invasives ou de bactéries. D'autre part, selon Lee (2001), l'injection graduelle de carbone organique en plus petite quantité réduirait le développement des contaminations comparativement à l'ajout initial d'une grande quantité. Bien que ces paramètres de stabilité de la culture ne soient pas les principaux sujets de la présente étude, ils doivent être pris en considération puisqu'ils influencent de façon importante la productivité du consortium. La productivité en biomasse et en lipides totaux visée dans le projet est respectivement de 0,4 $g \cdot L^{-1} \cdot jour^{-1}$ et 0,16 $g \cdot L^{-1}$ $jour^{-1}$. Les équipes de Alga-Labs, de RTA et de la CRIEB ont estimé qu'une productivité en biomasse de 0,4 $g \cdot L^{-1}$ $jour^{-1}$ et en lipides totaux de 0,16 $g \cdot L^{-1} \cdot jour^{-1}$ permettraient d'aller de l'avant avec des essais de démonstration à plus grande échelle si elles étaient atteintes.

1.4 Problématique

La concentration en nutriments dans les eaux usées et résidus de l'usine étant trop faible pour soutenir la productivité algale visée dans le projet, certains nutriments devront être ajoutés au milieu de culture. Le carbone organique, l'azote et le phosphore sont les plus susceptibles de devenir limitant pour la croissance des algues en fonction du mode de culture sélectionné. Pour atteindre les objectifs du projet, la détermination de leur concentration optimale pour la productivité de la souche s'impose.

D'autre part, des sources alternatives de nutriments doivent être envisagées pour subvenir aux besoins en carbone organique de la culture. Bien qu'il ait été démontré que le glucose est une source de carbone organique facilement assimilable par les algues (Xiong, Li *et al.*, 2008; Gao, Zhai *et al.*, 2010) et qu'il est celui qui procure le plus d'énergie sous forme d'ATP chez plusieurs espèces (Perez-Garcia, Escalante *et al.*,

2011), une source plus économique doit être utilisée pour assurer la rentabilité du projet. Certains résidus d'industries agroalimentaires pourraient avoir ce potentiel.

1.5 Besoin en nutriments des microalgues pour la production de biomasse et de lipides

Au niveau de la disponibilité en nutriments, le carbone est l'élément devant être le plus abondant dans le milieu puisqu'il forme le principal constituant des microalgues (40 à 50 % du poids sec). L'azote, un composant essentiel des protéines structurales et fonctionnelles, est le deuxième en importance (7 à 10 % du poids sec). Enfin, le phosphore est un autre nutriment majeur (environ 1 % du poids sec) qui joue un rôle important dans les processus métaboliques en formant plusieurs composés structurels et fonctionnels tels que l'ATP et les acides nucléiques (Richmond, 2008; Mata, Martins *et al.*, 2010; Barsanti et Gualtieri, 2014). Ces trois éléments sont les composants influençant majoritairement la croissance des algues. Ils sont généralement présents dans les algues vertes selon le ratio de C106 : N16 : P1, défini par Redfield (1958). Leur absorption est proportionnelle et advenant une carence en un de ces éléments, il devient un facteur limitant la croissance malgré la disponibilité des autres nutriments (Rhee et Gotham, 1980). Il s'avère que ce ratio, universellement accepté, serait toutefois variable selon les espèces et les conditions de cultures (Rhee et Gotham, 1980). D'autres auteurs ont aussi relevé une plasticité dans ce ratio, liée à la disponibilité de ces éléments (Geider et La Roche, 2002). En effet, l'absorption d'un élément par l'algue serait proportionnelle à sa concentration dans le milieu (Knauss et Porter, 1954). La concentration en carbone, azote, phosphore et leur ratio ont donc un impact majeur sur la productivité des algues ainsi que sur leur dominance dans une communauté planctonique.

D'autre part, il a été démontré qu'une limitation en azote favorise l'accumulation de lipides dans les cellules algales (Richmond, 2008; Xiong, Liu *et al.*, 2010; Gardner, Peters *et al.*, 2011; Mairet, Bernard *et al.*, 2011; Praveenkumar, Shameera *et al.*, 2012). La limitation en azote crée des conditions de stress qui stimulent la production de

substances de réserve sous forme de triacylgycérol (TAG). Néanmoins, une carence en azote limite la production de biomasse. Un équilibre entre la production en biomasse et la concentration cellulaire en lipides est alors essentiel pour atteindre une productivité optimale de lipides, cette dernière étant obtenue par leur produit mathématique (0.1).

$$(0.1)\,(g \text{ de biomasse} \times L^{-1}) \times (\text{mg lipides} \times g^{-1}) = \text{mg lipides} \times L^{-1}$$

Par exemple, Li, Yuan *et al.* (2011) ont réalisé un design expérimental (Box-Behnken) croisant trois différentes concentrations de carbone organique, d'azote et de phosphore. La productivité maximale de lipides de la souche *Chlorella minutissima* UTEX2341 a été obtenue dans les conditions procurant aussi une productivité maximale en biomasse, bien que la concentration cellulaire en lipides n'était pas la plus élevée. De la Hoz Siegler, Ben-Zvi *et al.* (2011) ont pour leur part réalisé un plan factoriel sur trois concentrations de carbone organique et d'azote. La productivité maximale de biomasse de la souche *Auxenochlorella protothecoides* UTEX25 a été atteinte avec la concentration médiane d'azote, mais aucune différence significative n'a été détectée entre les différents traitements en ce qui concerne la concentration de lipides. De plus, les concentrations de carbone organique expérimenté n'avaient aucune influence sur la production de biomasse et l'accumulation de lipides. Comme ces études le témoignent, les besoins en nutriments sont spécifiques à la souche de microalgues. Les conditions de culture vont également causer une variabilité. Il est donc essentiel de définir les besoins de chaque système de culture.

La quantité d'huile accumulée par la souche de microalgues revêt une importance pour la production de biocarburant, mais il est également nécessaire de considérer les types de lipides puisqu'ils vont influencer les propriétés du biocarburant. Ils auront entre autres un impact majeur sur la qualité si l'huile est transformée en biodiesel, car cette utilisation requiert une plus grande spécificité comparativement à la combustion de l'huile brute comme mazout. C'est d'ailleurs pourquoi les lipides contenus dans les cellules algales peuvent être séparés en deux groupes selon leur polarité : polaire ou neutre. Les phosphoglycérolipides, principales molécules composant les membranes, font partie du premier groupe. Les TAG s'intègrent dans le deuxième. Ils se composent

de trois molécules d'acides gras liées à une molécule de glycérol. Ce sont les TAG qui offrent le meilleur potentiel pour la fabrication de biodiesel en raison de leur plus forte proportion en acides gras et de l'absence de phosphate (Pruvost, Van Vooren *et al.*, 2009). La concentration en nutriment peut influencer le profil d'acides gras contenu dans les microalgues alors que ce même profil peut avoir un impact sur la qualité du biodiesel. En effet, la longueur des chaînes de carbones des acides gras, le nombre de doubles liaisons qu'ils contiennent avec un atome d'oxygène et leur positionnement vont influencer les propriétés telles que la densité, la viscosité, le point d'éclair et le pouvoir calorifique (Xu, Miao *et al.*, 2006). Moser et Vaughn (2012) mentionnent qu'un fort pourcentage d'acides gras mono-insaturés à 16 et 18 atomes de carbone (C16:1 et C18:1) constitue le profil idéal puisqu'il procure un équilibre entre la stabilité oxydative et les propriétés de tension à froid. Par ailleurs, Griffiths, Hille *et al.* (2011) ont démontré qu'une carence en azote pouvait tripler la proportion de C18:1 chez une espèce de *Chlorella vulgaris*. Plusieurs auteurs ayant étudié le profil lipidique et le rendement en huile de différentes souches affirment que certaines espèces de *Chlorella* posséderaient les caractéristiques adéquates pour produire du biodiesel comparable au diesel conventionnel (Xu, Miao *et al.*, 2006; Li, Xu *et al.*, 2007; Liu, Huang *et al.*, 2011). Mais comme il a été souligné, les conditions de nutrition doivent être étudiées puisqu'elles affectent la synthèse des lipides dans les cellules.

1.6 Source de carbone organique alternatif

Certaines espèces de microalgues ayant la capacité de croître en mixotrophie peuvent utiliser toute une variété de molécules de carbone organique autre que le glucose comme énergie chimique pour leur métabolisme cellulaire. Plusieurs types de résidus produits par le secteur agroalimentaire sont susceptibles de contenir des molécules carbonées potentiellement assimilables pas les microalgues. Ils pourraient ainsi constituer une source de carbone organique alternative pour une production mixotrophique de microalgues. Tel que défini par Tessier (2007), une source alternative contient des nutriments non conventionnels et souvent complexes, utilisés en

remplacement de composés plus simples dans un but de réduction de coûts. Un bénéfice environnemental en découle aussi grâce à la valorisation de résidus.

La croissance et les métabolismes impliqués dans des cultures alimentées par des composés alternatifs simples comme l'acétate et le glycérol ont été étudiés par plusieurs auteurs (Lee, 2001; Bouarab, Dauta *et al.*, 2004; Liang, Sarkany *et al.*, 2009; Heredia-Arroyo, Wei *et al.*, 2011; Rai, Nigam *et al.*, 2013). En ce qui concerne des sources de carbones plus complexes, des mélasses de divers types et des résidus d'industries agroalimentaires pourraient constituer des alternatives profitables. Par exemple Gao, Zhai *et al.* (2010) ont étudié la croissance d'une souche de *Chlorella protothecoides* dans un milieu de culture supplémenté en sirop de sorgho hydrolysé. Le sorgho est une plante africaine riche en saccharose, glucose et fructose. Des productivités en biomasse et en lipides de 1,2 $g \cdot L^{-1} \cdot j^{-1}$ et 0,6 $g \cdot L^{-1} \cdot j^{-1}$ ont été atteintes avec cette source de carbone comparativement à 0,74 $g \cdot L^{-1} \cdot j^{-1}$ et 0,4 $g \cdot L^{-1} \cdot j^{-1}$ avec du glucose. Des résultats similaires ont été obtenus sur cette même espèce par d'autres chercheurs qui ont comparé divers hydrolysats au glucose, comme Xu, Miao *et al.* (2006) avec la poudre de maïs hydrolysée et Wei, Zhang *et al.* (2009), avec de l'amidon de manioc hydrolysé. Yan, Lu *et al.* (2011) ont aussi obtenu une productivité en biomasse de 32 % supérieure au glucose avec des résidus de mélasse hydrolysés, mais une concentration cellulaire en lipides inférieure. La présence, entre autres, de fructose dans ces sources complexes pourrait expliquer la productivité en biomasse supérieure au glucose puisque *Chlorella protothecoides* aurait la capacité de métaboliser ce sucre (Rodríguez-López, 1966). D'autre part, le plus faible taux de lipides obtenu avec la mélasse s'expliquerait par leur contenu en azote. Finalement, EL-Sheekh, Bedaiwy *et al.* (2012) ont aussi démontré que la croissance de la souche *Chlorella vulgaris* avec un hydrolysat de matière lignocellulosique obtenu par hydrolyse enzymatique de son de blé était équivalente au glucose.

Le lactosérum de soja et la drêche sont d'autres résidus ayant été étudiés (Mitra, van Leeuwen *et al.*, 2012; Zhang, Su *et al.*, 2012). Le premier est un résidu produit lors de la fabrication du tofu et se compose de sucres solubles, d'azote, de phosphore,

de minéraux et d'éléments traces. Le second provient de la production de bioéthanol à partir du maïs et contient des sucres, des acides organiques, du glycérol, de l'azote et d'autres micronutriments. La productivité en biomasse obtenue par une souche de *Chlorella vulgaris* avec ces composés complexes a été respectivement de 1,6 $g \cdot L^{-1} \cdot j^{-1}$ et 2,5 $g \cdot L^{-1} \cdot j^{-1}$. En comparaison, elle a été de 2,0 $g \cdot L^{-1} \cdot j^{-1}$ avec le glucose.

Le lactosérum, un sous-produit laitier issu de la fabrication du fromage, a aussi été expérimenté comme source de carbone organique pour la production d'algues, bien que la littérature ne comporte que très peu d'études sur le sujet. Le lactosérum se compose principalement de lactose (4,5-5 % p/v), de protéines solubles (0,6-0,8 % w/v), de lipides (0,4-0,5 % p/v), de sels minéraux, dont le NaCl, le KCl, et des sels de calcium (8-10 % p/v), de l'acide lactique (0,05 % p/v), de l'acide citrique, de l'azote sous forme d'urée, du phosphore et des vitamines du groupe B (González, Cañizares *et al.*, 1997). Blier, Laliberté *et al.* (1996) ont obtenu une production en biomasse de 0,565 gL^{-1} après 16 jours de culture de la cyanobactérie *Phormidium bohneri* dans un milieu enrichi de lactosérum. Les résultats sont certes inférieurs à d'autres, présentés précédemment, mais ils pourraient s'expliquer par le fait que le but de l'étude était l'épuration d'azote et de phosphore et non l'atteinte d'une productivité maximale de biomasse ou de lipides à des fins énergétiques.

Freyssinet et Nigon (1980) ont quant à eux étudié l'effet de la poudre de lactosérum hydrolysée sur la croissance de *Euglena gracilis*. Leur conclusion est qu'elle ne stimulait pas assez la croissance pour générer une production industrielle de cette espèce. Mais ils mentionnent toutefois que des espèces appartenant au genre *Chlorella* et *Scenedesmus* pourraient éventuellement être utilisées à cette fin puisqu'elles ont la capacité de métaboliser le galactose et le lactose contenus dans le lactosérum sous certaines conditions de culture. Samejima et Myers (1958) rapportent en effet que le galactose, un monosaccharide issu de l'hydrolyse du lactose, pouvait soutenir la croissance hétérotrophique stricte de *Chlorella pyrenoidosa*.

Plus récemment, Abreu, Fernandes *et al.* (2012) ont également étudié l'utilisation de la poudre de lactosérum comme source de carbone organique. Ils ont comparé la croissance d'une souche de *Chlorella vulgaris* en photoautotrophie à celle obtenue en mixotrophie selon trois traitements : avec de la poudre de lactosérum non hydrolysée (nhPL), avec de la poudre de lactosérum hydrolysée (hPL) et avec un mélange de sucres pur glucose - galactose (GG). La consommation par les algues du glucose et du galactose a également été mesurée. La meilleure productivité en biomasse et en lipides ainsi que la meilleure efficacité d'assimilation des sucres a été obtenue avec la hPL. Les résultats de productivité (biomasse et lipides en $g \cdot L^{-1} \cdot j^{-1}$) ont été de 0,75 et 0,25 (hPL), 0,32 et 0,09 (nhPL), 0,46 et 0,14 (GG) et 0,10 et 0,04 en photoautotrophie. Les auteurs suggèrent l'hypothèse que le hPL contenait des nutriments supplémentaires pour expliquer sa supériorité face au glucose-galactose. L'acide lactique que contient le lactosérum en serait un exemple puisqu'il serait métabolisable par certaines espèces de *Chlorella* (Darley, Wimpee *et al.*, 1981; Tuchman, Schollett *et al.*, 2006). La même hypothèse expliquerait aussi pourquoi la nhPL, contenant du lactose plutôt que du glucose et du galactose, a permis une meilleure croissance qu'en photoautotrophie. Bien que la consommation du lactose n'ait pas été mesurée, ce composé pourrait aussi avoir eu un impact sur la croissance de la souche. Girard, Roy *et al.* (2014) ont par ailleurs étudié la dynamique de concentration de lactose dans un milieu de culture composé à 40 % de perméat de lactosérum. Leurs recherches ont révélé que la souche *Scenedesmus obliquus* avait la capacité d'hydrolyser le lactose pour ensuite assimiler le glucose et le galactose.

Le type de résidus utilisés comme source de carbone organique aura une influence sur la croissance des microalgues, mais également sur la production de lipides et le profil d'acides gras, comme c'est le cas pour la concentration en nutriments. L'étude de Mitra, van Leeuwen *et al.* (2012) mentionnée précédemment rapporte effectivement une variabilité dans la proportion des acides gras de la souche *Chlorella vulgaris* entre les milieux enrichis de glucose, de lactosérum de soya et de drêche. Il est donc nécessaire de tenir compte de cet aspect dans le choix de la source de carbone organique alternatif pour une production de microalgues.

1.7 Objectifs de l'étude

La présente étude a pour but d'élaborer d'un milieu de culture à base des eaux usées de l'usine Rio Tinto Alcan situé à Alma (Québec), en tenant compte des éléments soulevés dans la problématique, et ce, selon trois objectifs. Le premier objectif consiste à définir les concentrations de carbone organique, d'azote et de phosphore procurant une productivité maximale en biomasse et en lipides du consortium algues-bactérie constitué majoritairement de *Chlorella sp.* tout en limitant le développement de contamination biologique. L'utilisation de designs expérimentaux a permis de réaliser cet objectif dans un temps raisonnable, ce qui n'aurait pas été possible avec des expériences en triplicata. Le glucose a été la source de carbone organique utilisée pour ces expériences afin de limiter la variabilité causée par d'autres composés présents dans des sources plus complexes. Les concentrations définies ont pu ainsi servir de point de départ pour la réalisation du deuxième objectif.

L'étude de sources alternatives de carbone organique en remplacement du glucose constitue le deuxième objectif. Le choix de ces sources a été effectué en fonction de leur disponibilité, leur accessibilité et leur potentiel rapporté dans la littérature. Deux composés ont été sélectionnés : un hydrolysat cellulosique de résidus de maïs et un sous-produit laitier issus d'une usine de transformation de produits laitiers.

Le troisième objectif quant à lui consiste à évaluer la qualité de l'huile extraite pour une éventuelle transformation en biodiesel par l'analyse du profil des acides gras.

Cette étude s'insère dans un projet de plus grande envergure orienté vers un concept de cohabitation. Ce projet comporte des aspects uniques peu traités dans la littérature tels que l'utilisation d'un consortium algues-bactéries, culture dans les eaux usées d'une aluminerie et mixotrophie en bassin ouvert (milieu non stérile). La présente étude, qui aborde des éléments à la base de la production de microalgues, soit la nutrition, s'intègre ainsi dans un cadre original.

CHAPITRE II

MIXOTROPHIC CULTIVATION OF AN ALGAE-BACTERIA CONSORTIUM IN ALUMINIUM SMELTER WASTEWATERS (QUEBEC, CANADA): A HIGH CONCENTRATION IN NITROGEN INCREASES LIPID PRODUCTION

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Abstract

To produce energy for in-house use, an aluminium smelter in Québec launched a study of mixotrophic cultivation of microalgae in its wastewaters with the objective of having an algae production company set up operations on site. To maximize lipid productivity and maintain the biological integrity of the consortium, specific nutrients need to be added to aluminium smelter wastewaters to cultivate the selected

algae-bacteria consortium. A 2^3 factorial design was used to determine the organic carbon, nitrogen and phosphate strain needed. Data on biomass and lipid productivity, as well as a "consortium integrity index", were analysed using a multiple linear regression model. The highest biomass productivity (0.93 $g \cdot L^{-1} \cdot d^{-1}$) and lipid productivity (0.023 $g \cdot L^{-1} \cdot d^{-1}$) were obtained using the highest tested concentration in nitrogen (0.200 $g \cdot L^{-1}$) and the lowest tested concentration in phosphate (0.003 $g \cdot L^{-1}$). No significant effect of the organic carbon (glucose) concentration was detected in the range of test concentrations. To achieve maximal lipid production, the results suggested that biomass productivity should be prioritized rather than lipid accumulation in the cells through nitrogen starvation. The stability and integrity of the cultured consortium have to be maintained through an appropriate balance of nutrients. A low phosphate concentration increases the stability of the consortium, although part of the variation cannot be explained by the model. Finally, an analysis of fatty acid profiles showed that different concentrations in organic carbon, nitrogen and phosphorus impact the proportion of C18:1 (n-9) and other minor fatty acids.

Key words

Native algae-bacteria consortium, wastewater, nutrient balance, co-location, mixotrophy, biodiesel, consortium integrity index.

Introduction

In the current environmental and global energy context, aluminum producers are interested in using renewable sources of energy to reduce their environmental footprint and secure their energy supply. Production of rich lipid algal biomass is a potential alternative. Microalgae offer high productivity per surface unit (Chisti 2007) compared with other industrial plant crops and can be converted to valuable fuel or other forms of energy. Some examples of such renewable energy sources are the production of biogas via methanation (Golueke and Oswald 1959), direct combustion (Kadam 2002), ethanol production through the fermentation of microalgae sugars (John *et al.* 2011), biodiesel

production by oil extraction and transesterification (Mata *et al.* 2010), and biocrude oil production through hydrothermal liquefaction (López Barreiro *et al.* 2013).

On the other hand, microalgae production in co-location with an industrial plant can improve the profitability and environmental benefits of the overall process (Lohrey and Kochergin 2012; McGinn *et al.* 2011). Recycling of wastewater (Pittman *et al.* 2011) and other wastes that contain nutrients (Perez-Garcia *et al.* 2011) as well as the use of residual heat (Xu *et al.* 2011) and effluent gas (Rosenberg *et al.* 2011) from industrial processes can reduce microalgae production costs.

Many factors come into play in selecting the growth pattern and culture system. In the specific case of this study, the energy production objective and co-location with an aluminium smelter must be considered. Production under mixotrophic conditions appears to be the most convenient metabolic approach in this context. First, it promotes the fixation of industrial effluent CO_2 by photosynthesis (autotrophy) and reduces greenhouse gas emissions (Kadam 2002). Second, because photosynthesis may not always be optimal in areas such as Canada during the winter season, heterotroohic growth allows to overcome light limitation. The metabolization of organic carbon (heterotrophy) from industrial wastewaters and residues (nutrient wastes) and use of waste energy from the plant can maintain productivity year round while creating economic value from waste (Abreu *et al.* 2012). Mixotrophy could also be more effective in producing lipids than either heterotrophy or autotrophy (Mitra *et al.* 2012). The use of photo-bioreactors as a culture system can result in excessively high OPEX for the production of low-cost commodities such as biofuel. The use of open ponds could therefore be the best option (Resurreccion *et al.* 2012).

Carbon (C), nitrogen (N) and phosphorus (P) are major constitutive elements of almost all living organisms (Richmond 2008). For microalgae cultivation, these elements must be present in the culture medium in keeping with the needs of the specific strain. The concentrations of those nutrients will impact biomass productivity and lipid accumulation in the cells. It is known that addition of a sufficient amount of carbon and

nitrogen maximizes the production of biomass (Li *et al.* 2011), while nitrogen starvation triggers neutral lipid accumulation (Piorreck *et al.* 1984). The nutrient ratio also affects the fatty acid profile. For example, nitrogen deprivation increases the percent of oleic acid (C18:1 ω-9) in several species (Thomas *et al.* 1984). A balance between biomass production and cellular lipid concentration is essential to achieve optimal production of valuable lipids.

Mixotrophic microalgae cultivation in open ponds requires the right nutrients concentration and balance to ensure algae growth and control contamination from unwanted microorganisms (fungi, protists, rotifers, undesired bacteria and algae) that compete for food. Several ways to reduce competition and maintain the stability in algae cultures are reported in the literature: (1) increasing the microalgae inoculum size (Zheng *et al.* 2012), (2) use of a consortium rather than a pure strain (Bhatnagar *et al.* 2011), (3) addition of a small quantity of organic carbon by fed-batch rather than a large by batch (Lee 2001) and (4) alkaline pH of the culture medium. Koller *et al.* (2012) also observed that culture medium containing a high nutrients concentration limit the development of undesired microorganisms. Nutrients concentration and balance can thus affect microalgae productivity indirectly by increasing or reducing contamination.

In the context of this study, an algae-bacteria consortium, mainly *Chlorella sp.*, was isolated from aluminium smelter wastewaters. The consortium was cultivated under mixotrophic conditions, in the same wastewater, to obtain algal cells with a high lipid content. Because smelter wastewater does not contain enough nutrients to support optimal growth of the consortium, supplements were added. To keep low production cost, local industrial and agri-food wastes can be used to replace conventional nutrient sources. However, tests with conventional nutrients have to be done at the outset to determine the consortium's actual requirements for each element, and avoid any interference with other compounds.

The purpose of this study was to determine appropriate concentrations of the main nutrients (C, N and P) needed to achieve high lipid productivity and to observe the effect

they have on consortium biomass production. To compare the stability of the strain under particular conditions (C, N, P concentrations), an integrity index for the consortium was measured. The consortium integrity index is based on the proportion of *Chlorella sp.* in relation to the total number of observed microorganisms (including any undesired organisms). Fatty acid profiles were also analyzed to evaluate the effects of C, N and P concentrations on oil quality for further conversion into biodiesel by transesterification.

Materials and methods

Strain and culture

The algae-bacteria consortium cultivated in this study is a native strain, dominated by *Chlorella sp.* that was isolated in previous studies from aluminium smelter wastewater, the same as the aluminum smelter effluent-based culture medium used in this study. The seed culture was cultivated in an aluminum smelter effluent-based medium, to which Bold Basal medium nutrients were added (Bischoff and Bold 1963) with the following modifications: KNO_3 0.750 $g.L^{-1}$, KH_2PO_4 0.700 g/L, $MgSO_4 \cdot 7H_2O$ 0.300 g/L and sodium ferric EDTA 0.028 g/L. Glucose was added as an organic carbon source in fed-batch mode to achieve a final concentration of 1 $g.L^{-1}$ per culture cycle.

The inoculum used in this study was collected from a seed culture in exponential growth phase. It had a concentration of 5 x 10^7 cells ml^{-1} and represented a volume of 10% (v/v) of the medium. Cultures were grown in 1 L Erlenmeyer flasks containing 500 ml of medium. They were agitated using an orbital shaker at 130 rpm, under fluorescent lighting 12 h / day with an intensity of 20 µmol photons$\cdot m^{-2} \cdot sec^{-1}$ (measured by a LI COR Model LI-250 photometer, Lincoln, USA). The temperature was controlled at 21°C and the pH was measured on a daily basis (pH meter Symphony, SB70P model VWR, Radnor, USA).

The experiment was carried out with organic carbon (C), nitrogen (N) and phosphorus (P) at three different concentrations (see Table 1). For Mg and Fe, the same nutrient concentration as seed culture was added. The culture was grown for 9 days, including a 4-day acclimation period and a 5-day exponential growth period starting when glucose was injected. A second injection was done 48 h after the first one.

Biomass concentration and productivity

The Borowitzka and Moheimani (2013) method was modified and used to measure biomass concentration (dry weight). Ten millilitres of final culture were filtered on a previously weighted glass microfibre filter (Whatman 934-AH, porosity 1.5 µm) and washed with demineralized water. Filters were dried for 24 h at 60°C and weighted again.

Productivity was calculated for the exponential growth period according to the following equation (1):

(2) $P_b = (B_f - B_i)/T$

P_b = Biomass productivity ($g \cdot L^{-1} \cdot d^{-1}$)

B_f = Final biomass ($g \cdot L^{-1}$)

B_i = Initial biomass ($g \cdot L^{-1}$)

T = Total days of culture (culture period)

Neutral lipids concentration and productivity

Neutral lipids were extracted from the end culture biomass and quantified using a gravimetric method (De la Hoz Siegler Jr 2011). Microalgae biomasses from each culture were washed with demineralized water and vacuum dried with a Savant Speedvac System, model SS21 (Thermo Savant, Waltham, USA). 0.05 g per sample of dry biomass was crushed in liquid nitrogen, soaked overnight in 5 mL of hexane and ultra-sonicated (20 hz) using a sonifier with a microprobe (Sonifier 350 Cell Disruptor and

A3-561 microprobe from Branson Sonic Power Co., Danbury, USA) in order to break down algal cells. Cell fragments were separated from the liquid phase by centrifugation (5000 G, 10 min). Oil was weighed after complete evaporation of the solvent. Every measurement was completed in duplicate. Neutral lipid productivity was calculated as follow (2):

(3) $P_l = L \times P_b$

P_l = Neutral lipid productivity ($g \cdot L^{-1} \cdot d^{-1}$)

P_b = Biomass productivity ($g \cdot L^{-1} \cdot d^{-1}$)

L = Neutral lipids in g per g of biomass

Consortium integrity

Consortium integrity ensured strain stability. It is an important variable to measure because loss of strain stability can affect productivity. Consortium stability may be threatened by an undesired microorganism ratio. Algal cells were counted daily using a Neubauer chamber (hemocytometer) and a phase-contrast microscope (Axio Scope A1 from Zeiss, Toronto, Canada) in accordance with the method described by Guillard and Sieracki (2005). Undesired microorganisms, regarded as biological contaminants, i.e., algae other than *Chlorella sp.*, protists and rotifers, were also counted. Because the fungal hyphae could not be individually counted, their number was estimated by dividing the surface they covered on the Neubauer chamber by the average Chlorella surface area (20 μm^2) to produce an "algal cell equivalent" count.
The integrity index was calculated as follows (3):

(4) $I = 100 - \sum (C_i / A_i \times 100) / T$

I = Integrity index

C_i = Total number of biological contaminants in Neubauer chamber

A_i = Total number of consortium algae in Neubauer chamber

T = Total days of culture

i = Day of culture

Fatty acids profile

Fatty acids were analyzed with a GC-MS using the method proposed by Li *et al.* (2013). Dry biomasses used for the analysis were stored for one year at -20°C. 2.5 mL of a BF3: MeOH (14% V/V) solution were added to the previously extracted lipids and heated (65°C, 20 min) in closed tubes. One milliliter of saturated NaCl and 1 mL of hexane were then added to each tube. The tubes were shaken and centrifuged, and the supernatant was collected for analysis. An analysis of fatty acid methyl esters (FAME) was done using an Agilent (Santa Clara, USA) 7820A gas chromatograph with a DB-WAX capillary GC column (30 m, I.D. 0.25 mm, film thickness 0.25 mm) connected to a mass spectrometer (Agilent, model 5977E, Santa Clara, USA). During mass spectra registration, the injection temperature was 90°C, and helium was used as a carrier (10.5 PSI, 90°C). Electron ionization was 70eV, the frequency 2.5 readings per s, and the range 50-650 m/z. Temperature, initially 100°C, was increased by $10°C \cdot min^{-1}$ for 2 min, and by $5°C \cdot min^{-1}$ until it reached 250°C. FAME identification and quantification was done using mass spectra standard Supelco® 37 Component FAME Mix (Bellafonte, USA).

Experimental design and statistical analysis

SAS JMP 10.0 software (SAS Institute Inc., Cary, North Carolina, USA) was used to develop a 2^3 factorial design with 3 central points based on organic carbon (C), nitrogen (N) and phosphorus (P) factors (Table 1). Data for three response variables, i.e., biomass productivity ($g \cdot L^{-1} \cdot d^{-1}$), neutral lipid productivity ($g \cdot L^{-1} \cdot d^{-1}$) and consortium index integrity, were analyzed using multiple linear regressions with the same software. Finally, multiple correlation was used to analyze three more variables: the percent of lipids in biomass (% W/W), the initial pH and the final pH.

Results

Experimental design

To determine the concentrations of organic carbon, nitrogen and phosphorus that generate maximal productivity, tests with eleven combinations of factors (C/N/P) were run in accordance with a 2^3 factorial design, including 3 central points (tests 2, 3 and 4; Table 2). These central points were used to measure the experimental error and to validate the models. The models were performed on response variables for biomass productivity, neutral lipid productivity and consortium index integrity, to determine whether ideal concentrations for lipid productivity also favoured biomass production and consortium stability (Table 2). Because the design central points for N and P were not exactly the middle value of the two levels, confusion in terms might have occurred. However, values in the alias matrix were 0 or close to it, showing that there was no confusion among terms (Table 3).

Biomass productivity

Results of the multiple linear regressions performed on the algae-bacteria consortium biomass productivities are shown in Table 4. Main variations are on the N and P factors. The parameter estimated for C was not significant ($p=0.10$, data not shown), and this factor was excluded from the model. The highest biomass productivity under our test conditions was 0.93 $g \cdot L^{-1} \cdot d^{-1}$ using 0.200 $g.L^{-1}$ nitrogen and 0.003 $g.L^{-1}$ phosphorus. Increasing of nitrogen concentration increased biomass productivity, whereas, increasing the phosphorus concentration decreased it (Fig. 1).

Lipids productivity

In the range of nutrient concentrations tested (Table 1), nitrogen and phosphorus were the main factors that affected lipid productivity as well as the linear interaction between these two factors (Table 5). The parameter estimated for C was again not significant ($p=0.10$, data not shown), and C was excluded. The highest lipid productivity

in our tests conditions was 0.023 $g \cdot L^{-1} \cdot d^{-1}$ using 0.200 $g.L^{-1}$ nitrogen and 0.003 $g.L^{-1}$ phosphorus (Fig. 2).

Integrity index

Under test conditions, the algae-bacteria consortium had a higher integrity index when P concentration was low. Phosphorus was the only predictor used because the parameter estimates for C and N were not significant (data not shown) (Table 6). Therefore, consortium integrity decreases or increases with phosphorus concentration (Fig. 3).

Correlations

The correlation matrix (Table 7) presents the relationship with others variables in addition to those included in the model (factors and responses). This analysis gives more information on relationships between responses and the indirect effects of nutrient concentration on the consortium, such as the variation in pH. Higher correlation coefficients are in bold. As expected, lipid accumulation in cells was negatively correlated to nitrogen concentration (-0.80), while biomass productivity was positively correlated (0.77) to nitrogen concentration. The consortium lipid and biomass productivity were highly correlated (0.94). Also, a positive correlation between initial pH and the integrity index was shown (0.71), as well as a negative correlation between final pH and neutral lipids in biomass (0.95).

Fatty acids profile

The fatty acid composition of neutral lipids from extracted oil affects the quality of biodiesel produced from this oil. The fatty acid profile from each culture was analyzed to show the effect of different concentrations of organic carbon, nitrogen and phosphorus on the proportion of fatty acids, and consequently the biodiesel conversion potential of the oil produced during each treatment. The main fatty acids found were

palmitic (C16:0), stearic (C18:0), and oleic (C18:1 n-9) acids (Fig. 4). Most fatty acids were saturated (57% to 91%), followed by mono-unsaturated (6% to 25%) and poly-unsaturated (<1% to 31%).

A multiple linear regression analysis of the fatty acids whose concentrations exceeded 1% in more than 1 treatment was performed using 3 factors; C, N and P. Fatty acids in low concentration, except oleic acid, were the most affected (Fig. 4 and Table 8). N was the more significant factor: a higher N concentration increased the proportions of lauric (C12:0), myristic (C14:0), margaric (C17:0), and heptadecenoic (C17:1) acids in total fatty acids and decreased the proportion of oleic (C18:1 (n-9)) acid. Interaction between C and N negatively affected the proportions of pentadecylic (C15:0) and heptadecanoic (C17:1) acids. Increasing P concentration decreased the proportion of pentadecylic (C15:0) acid and increased the proportion of heptadecenoic (C17:1) and oleic (C18:1 (n-9)) acids. Maximal variation among the treatments was 12% for oleic (C18:1 (n-9)) acids.

Discussion

Lipids and biomass productivities

The productivity of the selected algae-bacteria consortium cultivated in aluminium smelter wastewaters was directly affected by the nutrient concentrations in the culture medium. Results from a 2^3 factorial design shows that among the concentrations used, the maximal concentration of N (0.200 $g \cdot L^{-1}$) and the minimal concentration of P (0.003 $g \cdot L^{-1}$) resulted in the highest biomass (0.93 $g \cdot L^{-1} \cdot d^{-1}$) and lipid (0.023 $g \cdot L^{-1} \cdot d^{-1}$) productivity.

Under the conditions described, no effect of organic carbon on consortium productivity could be demonstrated. Very few studies have been performed on algal mixotrophic production in wastewaters other than agri-food wastewaters. Bhatnagar *et al.* (2011) reported biomass productivity of 0.215 $g \cdot L^{-1} \cdot d^{-1}$ using a

Chlorella minutissima strain cultivated in enriched N ($0.041 g \cdot L^{-1}$ g N from $NaNO_3$) and C ($0.4 g L^{-1}$ C from glucose) wastewaters from a carpet factory. Althought lipid productivity was not reported in this study, another article from same authors reported a lipids productivity of $0.004 g \cdot L^{-1} \cdot d^{-1}$ under the same culture conditions, without enrichment in N and C (Chinnasamy *et al.* 2010).

Negative productivities, due to biomass loss, were seen in two treatments with low N concentrations (Table 2). Lipid productivity depends on 2 parameters: biomass productivity, favoured by nitrogen, and lipid accumulation in cells, reduced by nitrogen (Illman *et al.* 2000). An equilibrium must be determined; conditions for highest lipid accumulation per cell could reduce total biomass (and the number of cells), leading to reduced total lipid production. In our study, the algae-bacteria consortium produced more lipids per day in conditions with high nitrogen concentration, although lipid accumulation in cells (proportion of neutral lipid cells) was negatively correlated to nitrogen concentration (Table 7). The correlation between lipid productivity and biomass was higher than the correlation between lipid productivity and lipid accumulation, thereby demonstrating that biomass productivity has a greater impact on lipid productivity than lipid accumulation. Li *et al.* (2011) came to the same conclusion using the strain *Chlorella minutissima* UTEX2341; lipid productivity was higher when culture conditions were optimized for biomass production, although lipid accumulation per cell was not maximal. Moreover, nitrogen has a greater impact on lipid accumulation than organic carbon (Table 7), as previously noted in the literature: Heredia-Arroyo *et al.* (2011) observed that the organic carbon concentration had no effect on the proportion of lipids in strain *Chlorella vulgaris* 2714 cells.

As shown in figures 1, 2 and 3, adding nutrients is essential to ensure consortium productivity in aluminium smelter wastewaters, but the impacts are different. While carbon is the major constituent of cells, its impact on biomass and lipid productivity was not significant. Models including the C factor were significant at α 0.05 threshold (data not shown), but the parameter estimate of this factor was significant at α 0.10. The culture trophic mode (mixotrophy) could explain those results.

In the presence of light, microalgae could photosynthesize using CO_2 from the air and bacterial respiration, in addition to metabolizing organic carbon. Thus, the predictor used (organic carbon) could not solely explain the productivity variability because both carbon supplies (CO_2 and organic carbon) are involved in consortium growth. Martínez and Orús (1991) have studied this aspect. They noted that the *Chlorella vulgaris* UAM 101 growth rate under mixotrophic conditions (light+CO_2+glucose) was the sum of the heterotrophic (glucose in darkness) and autotrophic (light+CO_2) growth rates.

The maximal organic carbon concentration could also be too low to have a significant effect on productivity. Adding runs to experimental design, with higher nutrient concentrations, could provide higher productivity and allows the detection of a quadratic relationship. For exemple, the response surface analysis in a study by De la Hoz Siegler et al. (2011) showed that the growth rate of the *Auxenochlorella protothecoides* strain decreases in high concentrations of organic carbon and nitrogen. It is possibly due to an inhibitory effect caused by excess substrate.

In addition to nutrients, pH can have an effect on lipid and biomass productivity. The biomass of *Chlorella protothecoides* can be increased by 16% if cultivated at pH 6 rather than pH 8 (Shi *et al.* 2006). In the present study, pH and biomass production are not strongly correlated (Table 7). Lipid metabolism and accumulation are known to be impacted by pH. High pH stimulated triacylglycerol accumulation in *Chlorella* CHLOR1 strain cells (Guckert and Cooksey 1990). However, in this study, the lipid accumulation in cells is inversely proportional to the final pH (R -0.95), and there is no correlation with the initial pH (Table 7).

Integrity index

Pure cultures of microalgae in open ponds are known to be easily contaminated by predators and competitive micro-organisms (Amaro *et al.* 2011) unlike algal-bacterial consortia that are considered more stable (Safonova *et al.* 2004). Consortium ecology is affected by culture conditions, including some nutrient concentrations (Rhee and

Gotham 1980). The determination of nutrient concentrations that maintain consortium integrity is essential for sustaining a high yield. In this study, consortium integrity is mainly affected by phosphorus concentration (Table 6 and Fig. 3). The correlation results (Table 7) lead us to assume that this effect could be related to the negative impact of phosphorus on pH. Furthermore, consortium integrity has only a slight effect on biomass productivity as demonstrated by the weak correlation with these variables (Table 7). The integrity index was however significantly correlated with lipid productivity (Table 7). Some contaminants such as fungal hyphae could contribute to biomass but less to lipid productivity owing to the fact their lipid content is lower than that of algae.

Fatty acid profile

Biodiesel can be produced from microalgae oil. Its properties depend on the algae fatty acid composition. The degree of unsaturation and chain length of fatty acids are the two main factors to be considered in estimating the quality of a biodiesel made from microalgal oil (Stansell *et al.* 2012). A large proportion of saturated and mono-unsaturated fatty acids and a small amount of poly-unsaturated fatty acids are required to produce a biodiesel that meets European and American standards. Regarding chain lengths, the carbon number has to range from 8 to 18 C. In the present study, carbon chains lengths varied mainly from C12 to C18, and less than 1% were long chains (C20 and more) (Fig. 4). According to Ramos *et al.* (2009), the oil produced by the consortium could have an oxidative stability and combustion properties comparable to conventional diesel.

Nutrients such as N and P could support or reduce the production of certain fatty acids. C18:1 (n-9) showed the strongest response to N (estimate = -3.2482) and P (estimate = 2.9538) variations (Table 8). It has been previously noted that a lower nitrogen concentration enhanced the production of C18:1 (n-9) (Griffiths *et al.* 2011; Stansell *et al.* 2012), but the phosphorus effect found in this study has not been documented in the literature. The fatty acids affected by N and P were in such a low

concentration in cells that they would have no effect on the quality of biodiesel produced.

Conclusion

In this study, a native consortium of algae and bacteria, mainly *Chlorella sp*, was cultivated in wastewaters from an aluminium smelter using CO_2 and organic carbon as sources of C (mixotrophy). High nitrogen and low phosphorus quantities (as compared with BBM) had to be added to these waters to enhance lipid and biomass productivity. It was shown that the culture conditions that enhanced biomass production resulted in higher lipid productivity than those that enhanced lipid accumulation in cells by reducing nitrogen. Consortium integrity was better preserved under these conditions (high N, low P), while high P promoted higher contamination of the consortium by unwanted micro-organisms. Despite the variation in the proportion of fatty acids in relation to N and P, nutrient concentrations did not drastically effect algal oil quality and biodiesel production potential.

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Tables

Table 1: Factors and levels of the 2^3 factorial design

Factors	Levels (g/L)		
	-1	0	1
C	0.657	1.057	1.457
N	0.020	0.100	0.200
P	0.003	0.053	0.200

Table 2: 2^3 Factorial design matrix with observed values

Trials	Factors			Biomass productivity ($g \cdot L^{-1} \cdot d^{-1}$)	Neutral lipids productivity ($g \cdot L^{-1} \cdot j^{-1}$)	Integrity index (%)
	C	N	P			
1	0.657	0.200	0.003	0.460	0.010	95.4
2	1.057	0.100	0.053	0.120	0.002	82.2
3	1.057	0.100	0.053	0.590	0.022	74.8
4	1.057	0.100	0.053	0.330	0.006	88.4
5	0.657	0.020	0.003	0.000	0.000	86.3
6	1.457	0.020	0.200	-0.450	-0.059	72.2
7	1.457	0.020	0.003	0.070	0.009	91.7
8	1.457	0.200	0.200	0.520	0.008	79.1
9	1.457	0.200	0.003	0.940	0.028	78.0
10	0.657	0.020	0.200	-1.150	-0.091	65.8
11	0.657	0.200	0.200	0.600	0.007	73.3

Table 3: Aliases matrix

	NC	CP	NP
Int[a]	0	0	0.014
C	0	0	0
N	0	0	-0.002
P	0	0	-0.007

[a] Int: Intercept.

Table 4: Model of consortium biomass productivity

Summary of fit					
R^2				0.77	
R^2 adjusted				0.71	
Root mean square error				0.31	
Mean response				0.18	
Observations				11	
ANOVA					
Source	D f[a]	Sum of squares	Mean squares	F ratio	Prob.>F
Model	2	2.5820	1.2910	13.4088	0.0028
Error	8	0.7702	0.0963	-	-
Total	10	3.3523	-	-	-
Parameter estimates					
Term	Estimate	Standard error	t ratio	Prob.>|t|	
Intercept	0.1645	0.0947	1.74	0.121	
N (0,02-0,2)	0.5017	0.1095	4.58	0.002	
P (0,003-0,2)	-0.2637	0.1063	-2.48	0.038	

[a] Df: Degree of freedom.

Table 5: Model of consortium lipids productivity

Summary of fit					
R^2				0.91	
R^2 adjusted				0.86	
Root mean square error				0.0013	
Mean response				-0.005	
Observations				11	
ANOVA					
Source	D f[a]	Sum of squares	Mean squares	F ratio	Prob.>F
Model	3	0.0118	0.0039	22.3417	0.0006
Error	7	0.0012	0.0002	-	-
Total	10	0.0130	-	-	-
Parameter estimates					
Term	Estimate	Standard error	t ratio	Prob.>|t|	
Intercept	-0.0080	0.0040	-1,97	0.089	
N (0,02-0,2)	0.0240	0.0047	5.12	0.001	
P (0,003-0,2)	-0,0240	0.0045	-5.29	0.001	
N*P	0.0171	0.0047	3.65	0.008	

[a] Df: Degree of freedom.

Table 6: Model of consortium integrity index

Summary of fit					
R^2				0.56	
R^2 adjusted				0.51	
Root mean square error				6.35	
Mean response				80.64	
Observations				11	
ANOVA					
Source	D f[a]	Sum of squares	Mean squares	F ratio	Prob.>F
Model	1	461.3100	461.310	11.4543	0.008
Error	9	362.4667	40.274	-	-
Total	10	823.7767	-	-	-
Parameter estimates					
Term		Estimate	Standard error	t ratio	Prob.>|t|
Intercept		79.6582	1.9354	41.16	<0.0001
P (0,003-0,2)		-7.3563	2.1736	-3.38	0.008

[a] Df : Degree of freedom.

Table 7: Pearson product moment correlation of factors and other mesured variables

	C[a]	**N[b]**	**P[c]**	**L. p[d]**	**B. p[e]**	**I. i[f]**	**% L[g]**	**In pH[h]**	**Fi pH[i]**
C[a]	1	0	0	0.19	0.23	0	0.31	0.04	-0.43
N[b]	-	1	0.01	0.59	**0.77**	0.12	**-0.80**	0.15	**0.68**
P[c]	-	-	1	**-0. 61**	-0.41	**-0.75**	0.11	**-0.92**	0.2
L. p[d]	-	-	-	1	**0.94**	0.57	-0.58	0.56	0.49
B. p[e]	-	-	-	-	1	0.38	**-0.64**	0.44	0.55
I. i[f]	-	-	-	-	-	1	-0.15	**0.71**	0.1
% L[g]	-	-	-	-	-	-	1	-0.07	**-0.95**
In pH[h]	-	-	-	-	-	-	-	1	0,14
Fi pH[i]	-	-	-	-	-	-	-	-	1

[a] Organic carbone;
[b] Nitrogen;
[c] Phosphorus;
[d] Lipids productivity;
[e] Biomass productivity;
[f] Integrity index;
[g] Neutral lipids accumulation in cells;
[h] Initial pH;
[i] Final pH.

Table 8: Models of consortia fatty acids (present in concentration more than 1% in more than one test) according to the C, N, P factors

	Adjustment		ANOVA						Parameter estimates				
Fatty acids	**R^2**	**R^2 ajust**	**Source**	**D f[a]**	**Sum of squares**	**Mean squares**	**F ratio**	**Prob.>F**	**Term**	**Estimate**	**Standard Error**	**T ratio**	**Prob.>|t|**
	0.49	0.43	**Model**	1	2.5212	2.5212	8.6924	0.016	**Int.**	0.9374	0.1625	5.77	0.0003
C12:0			**Error**	9	2.6104	0.2900			**N**	0.5604	0.1901	2.95	0.016
			Total	10	5.1316								
	0.55	0.50	**Model**	1	19.8961	19.8961	11.2238	0.008	**Int.**	3.2256	0.4017	8.03	<0.0001
C14:0			**Error**	9	15.9539	1.7727			**N**	1.5744	0.4699	3.35	0.008
			Total	10	35.8500								
	0.80	0.66	**Model**	4	1.1776	0.2944	5.8791	0.028	**Int.**	0.7630	0.6828	11.17	<0.0001
C15:0			**Error**	6	0.3005	0.0501			**C**	0.0711	0.0791	0.90	0.4031
			Total	10	1.4781				**N**	-0.0374	0.0790	-0.47	0.6524
									P	-0.2861	0.0766	-3.73	0.0097
									C*N	-0.2306	0.0791	-2.91	0.0268
	0,61	0.57	**Model**	1	0.6864	0.6864	14.0857	0.004	**Int.**	0.8031	0.0666	12.06	<0.0001
C17:0			**Error**	9	0,4386	0.0487			**N**	0.2924	0.0779	3,75	0,0045
			Total	10	1.1250								
	0.98	0.97	**Model**	5	12.3889	2.4777	62.9416	0.0002	**Int.**	1.4192	0.0605	23,44	<0,0001
C17:1			**Error**	5	0.1968	0.0394			**C**	-0.1719	0.0701	-2,45	0,0579
			Total	10	12.5854				**N**	0.9602	0.0700	13,71	<0,0001
									P	0.4875	0.0680	7,17	0,0008
									C*N	-0.1945	0.0701	-2,77	0,0393
									N*P	0.5425	0.0701	7,74	0,0006
	0.63	0.55	**Model**	2	156.7826	78.3913	7.0335	0.017	**Int.**	9.4186	1.0187	9,25	<0,0001
C18:1 (n-9)			**Error**	8	89.1634	11.1454			**N**	-3.2482	1.1785	-2,76	0,0248
			Total	10	245.9461				**P**	2.9538	1.1436	2,58	0,0325

[a] Df: Degree of freedom.

Captions and figures

Captions

Fig. 1

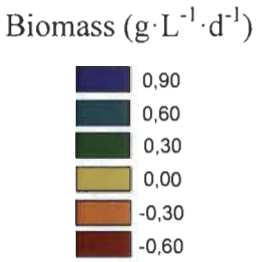


Fig. 2

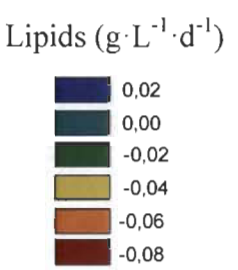
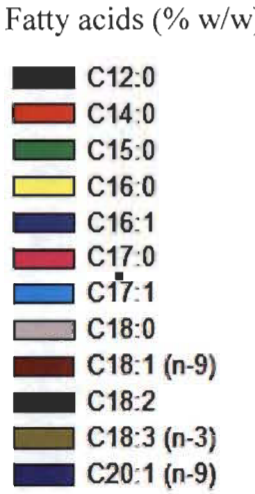


Fig. 4



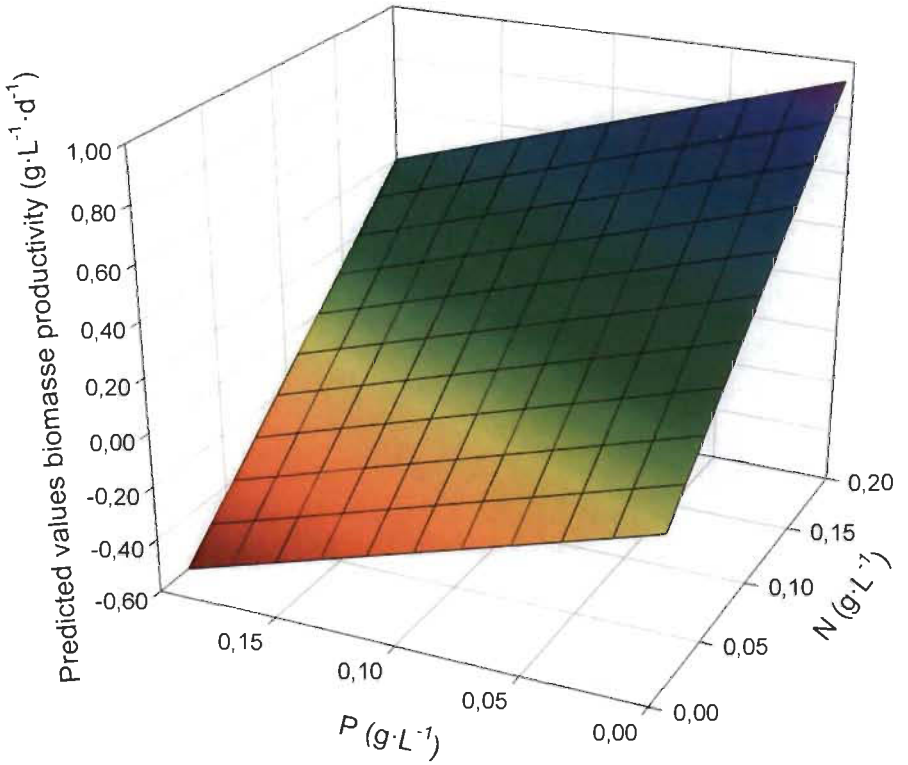


Figure 1: Surface profiler of the model of consortia biomass productivity according to nitrogen (N) and phosphorus (P) concentrations.

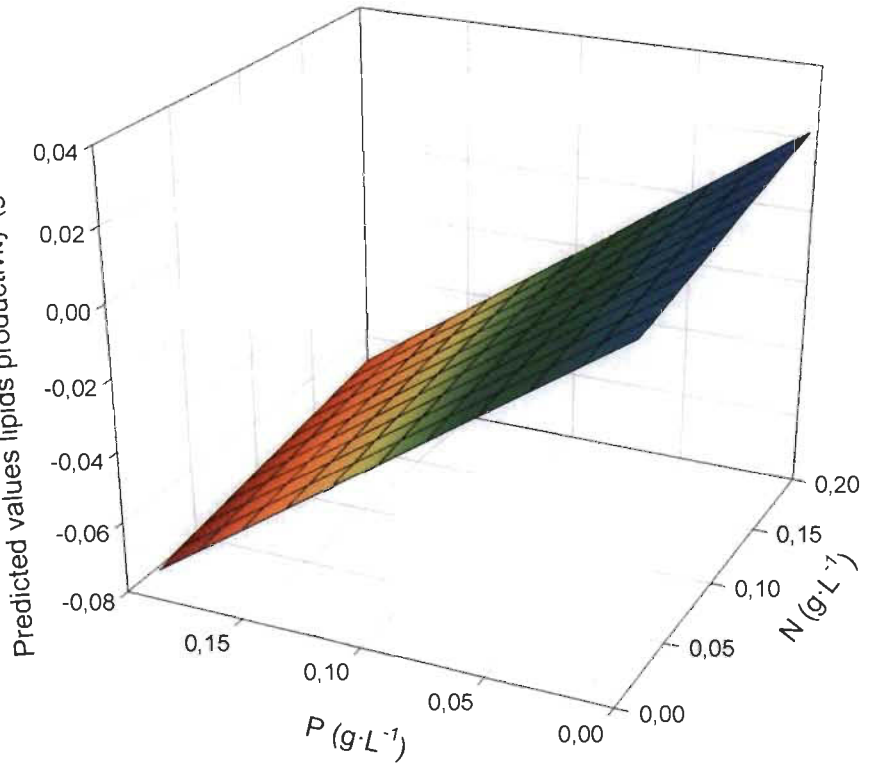


Figure 2: Surface profiler of the model of consortia lipids productivity according to nitrogen (N) and phosphorus (P) concentrations.

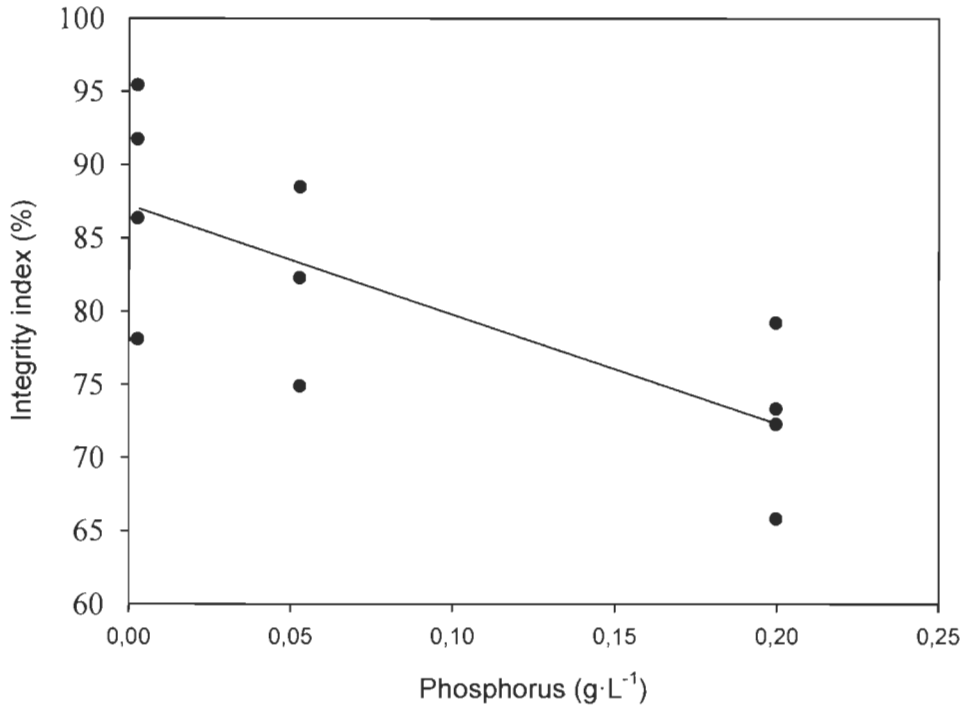


Figure 3: Linear regression of the model of consortia integrity index according to phosphorus concentration.

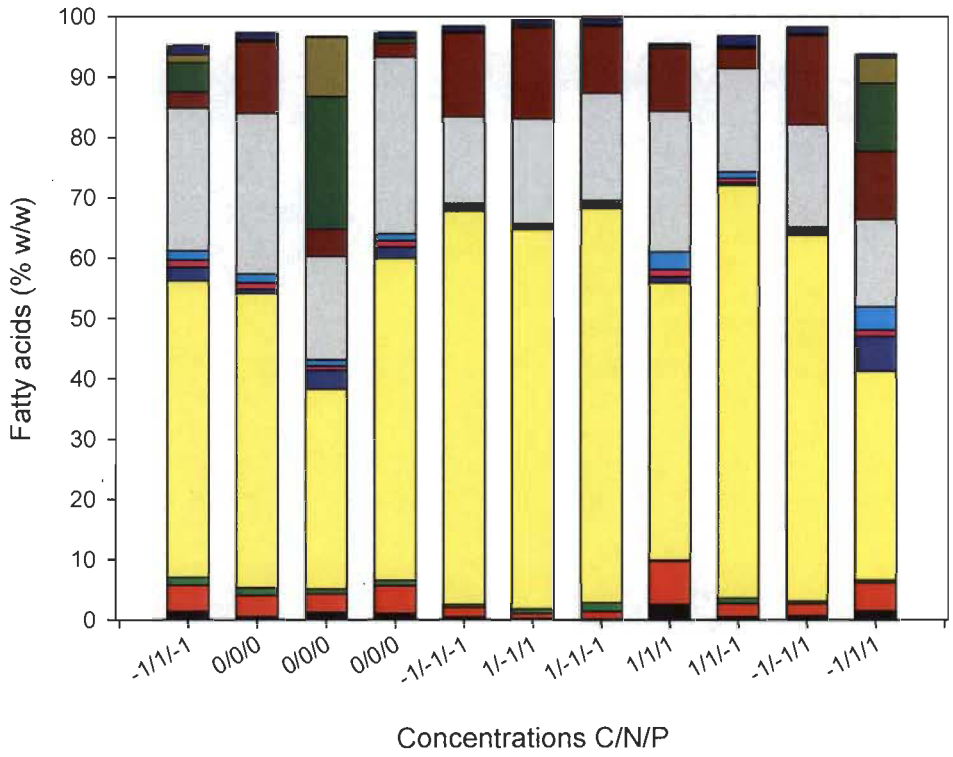


Figure 4: Fatty acids proportions (present in concentration more than 1% in more than one test) in the extracted lipids of design trials.

Le chapitre qui suit comporte un article présentant des travaux réalisés avec une souche différente de celle utilisée pour les travaux présentés au chapitre II. La stabilisation d'une souche est une étape essentielle, particulièrement dans le cas du mode de culture employé dans ce projet (mixotrophie dans des eaux usées non stériles). Elle nécessite plusieurs cycles de culture, ce qui peut s'étendre sur plusieurs mois voire plus d'une année. Toutefois, en raison de contraintes de temps liées au programme de recherche et au programme d'étude, les travaux doivent être lancés en parallèle à la stabilisation de la souche.

Dans le présent projet, la souche CHRT-A a préalablement été isolée des eaux usées par la compagnie Alga-Labs, collaboratrice du projet RTA. Elle a été maintenue en culture dans un milieu à base des eaux usées RTA additionnées de glucose et de nutriments selon le milieu standard Bold's basal. Cette souche a fait l'objet de l'étude présentée au chapitre II qui était la première étape (objectif 1) d'une suite logique : déterminer la concentration en nutriments (C, N, P) procurant une productivité maximale. La deuxième étape (objectif 2) consistait à remplacer un nutriment, soit le carbone organique, par une source alternative dans un but de réduction de coût de production de la biomasse algale. Les conditions de culture expérimentées ont cependant après quelques mois rendues la souche instable avant le début des travaux de la deuxième étape. Les micro-organismes compétiteurs ont fini par dominer la *Chlorella sp.* qui composait majoritairement le consortium. Cette souche ne convenait donc pas au mode de culture préconisé dans le projet.

Entre temps, une autre souche, la CHRT-B, avait été isolée par cette même compagnie, et était depuis peu maintenue en culture dans les mêmes conditions que la CHRT-A. Les travaux sur les concentrations en nutriments (C, N, P) ont été repris avec cette souche. Par contre, aucun modèle n'a pu être réalisé avec les résultats du design expérimental. En effet, la souche CHRT-B, nouvellement isolée, n'était pas encore stable et se faisait dominer par certains micro-organismes compétiteurs lorsque les concentrations en nutriments étaient modifiées. Certaines tendances ont tout de même pu être dégagées des résultats. Les travaux devant se poursuivre, les concentrations en

nutriments ont été fixées selon ces observations pour réaliser les expériences de l'objectif 2 sur les sources alternatives de carbone organique avec la souche CHRT-B.

CHAPITRE III

USE OF ALTERNATIVE ORGANIC CARBONE SOURCES IN MIXOTROPHIC CULTURE OF ALGAE-BACTERIA CONSORTIUM USING INDUSTRIAL WASTEWATERS

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Abstract

Rio Tinto Alcan (RTA), an aluminum production company, conducted a study to evaluate the feasibility of microalgae cultivation under mixotrophic conditions in their wastewaters with the aim of producing biofuel, to replace some of the fossil resources consumed by the plant. This colocation approach of microalgae production with an existing facility improves the profitability of the process. Costs can be reduced even further if agri-food residues are used as nutrients to compliment the nutrients found in

the smelter wastewaters. The potential of two alternative organic carbon sources to support growth and lipid production of an algal-bacterial consortium was then studied. One is a byproduct from a dairy product processing plant (DBP) and the other is cellulose hydrolysate (CH) obtained from corn crop residues. They were compared to glucose. Phototrophy was also used as control conditions in order to evaluate the productivity gain on mixotrophy. The biomass productivity obtained was as followed: DBP 0.47 ± 0.04 $g \cdot L^{-1} \cdot j^{-1}$, glucose 0.45 ± 0.15 $g \cdot L^{-1} \cdot j^{-1}$, CH 0.32 ± 0.06 $g \cdot L^{-1} \cdot j^{-1}$, and the control (phototrophy) 0.17 ± 0.01 $g \cdot L^{-1} \cdot j^{-1}$. In descending order, the neutral lipids content in cells (% p/p) was 3.0 ± 0.4% for the control (phototrophy), 2.0 ± 0.4% for glucose, 1.8 ± 0.2% for the DBP and 1.6 ± 0.2% for CH. No significant difference on biomass productivity and on neutral lipids content in cells was detected between the different sources of organic carbon. However, the control under phototrophy obtained a significantly lower biomass productivity, but a significantly higher neutral lipids accumulation. The integrity of the consortium has been preserved in all tested conditions. Finally, the carbon source (organic or inorganic) has little influence on neutral lipids profile of the studied strain. In regards of the results, DBP and CH could be used as carbon source in replacement of glucose for the mixotrophic growth of the studied algae-bacteria consortium.

Key words

Algae-bacteria consortium, wastewater, mixotrophy, dairy by-product, cellulosic hydrolysate, biodiesel.

Introduction

Because of a possible depletion of fossil resources, many industries are searching for reliable energy supplies for the future. As the starting point for the research on renewable sources, this objective intersects the current environmental concerns. Intensive microalgae cultivation for energetic purposes seems to be a promising avenue to meet these needs through biomass productivity and lipid content (Mata *et al.* 2010). For example, the yield of microalgae oil in $g \cdot m^{-2}$ is 10 to 20 times higher than palm oil

(Chisti 2007). Indeed, various types of fuels can be produced from algal biomass, for example: fuel from dried biomass (Pittman *et al.* 2011), crude oil through hydrothermal liquefaction (López Barreiro *et al.* 2013), biodiesel from extracted oil (Demirbas and Fatih Demirbas 2011), and methanization of biogas from waste biomass (Chisti 2007; Pittman *et al.* 2011; Singh and Gu 2010).

The implementation of integrated algae culture concepts in existing facilities is a development avenue facilitating the profitability of biofuel production (Lohrey and Kochergin 2012; McGinn *et al.* 2011). For example, reduction of the production costs related to the algae culture can be achieve by (1) recycling of wastewaters (Pittman *et al.* 2011) or residues containing nutrients (Perez-Garcia *et al.* 2011) (2) and the use of residual heat and gaseous effluents from plant production processes (Brennan and Owende 2010; Hundt and Reddy 2011; Rosenberg *et al.* 2011; Xu *et al.* 2011).

Rio Tinto Alcan, an aluminum production company, has undertaken a study on the production of microalgae through colocation with one of its plants in Quebec (Canada) and on the use of algal biomass to replace some of fossil energy used in the same facility. An algae-bacteria consortium composed mainly of *Chlorella sp.* was isolated from the smelter's wastewaters in order to produce algal biomass under mixotrophic conditions and in open tank.

Compared to phototrophy (CO_2 intake and light) and to heterotrophy (organic carbon intake), mixotrophy (CO_2 intake, light and organic carbon) would be the most productive mode for lipid production in some *Chlorella sp* (Mitra *et al.* 2012). Organic carbon intake would also palliate photosynthesis under low light intensity. In open tank, the competition control for substrate by undesirable microorganisms (fungi, protozoa, bacteria) and predation (rotifers), however, must receive a special attention. A concentrated inoculum (Zheng *et al.* 2012), a consortium rather than a pure strain (Bhatnagar *et al.* 2011), a fed-batch organic carbon intake (Lee 2001) and alkaline pH are means that can be used to limit this competition and maintain the stability of a culture of this type.

However, as the smelter's wastewaters are poor in nutrients (Bourdeau in preparation), agri-food residues shall be added to the medium to maximize the strain growth, while maintaining low production costs. As a possible source of organic carbon, two compounds were selected considering their potential, which was reported in literature, as well as availability. The first is a cellulosic sugar from the enzymatic hydrolysis of corn crop residues (CH).

Of the 991,000 hectares of cropland in Quebec in 2014, 42% was dedicated to corn (Quebec Statistic Institute 2014). Residues not usable for food or surplus of soil amendment (stems, stalks, leaves) are an important source of biomass that can be valued into sugar. To date, very few study reports the use of cellulosic sugar from corn residues as an organic carbon source for mixotrophic cultures of microalgae. However, US company Solazyme holds a patent on the heterotrophic culture of a Chlorella sp. with a cellulosic hydrolyzate mainly composed of glucose and xylose (Trimbur *et al.* 2009). In addition, a study by El-Sheekh *et al.* (2012) dealing with cellulosic hydrolysate of wheat demonstrated that the growth rate of a *Chlorella sp.* with this compound was almost equivalent to that obtained with glucose.

The second residue considered by RTA is a byproduct, containing organic carbon, which is from a manufacturing dairy plant (DBP). Unlike lactoserum, no recovery method is currently available for this residue. Quebec had 103 manufacturing dairy plants on its territory in 2013 (Canadian Government 2014). A significant quantity of this residue type would be available as an organic carbon source. Indeed, this residue was selected because of its abundance in the area of the aluminum smelter involved in the study. A very few studies dealing with the use of dairy byproducts out of lactoserum in mixotrophic microalgae cultures have been published. Dairy industry wastewaters have been already studied as a culture medium for phototrophic growth of *Chlorella pyrenoidosa* strain (Pathak *et al.* 2013). These wastewaters could achieve higher growth than in a standard medium.

Furthermore, different kind of fuel could be produced from algae biomass. One of them is biodiesel, obtained by the transesterification of fatty acids contents in cells lipids extracted. The type of fatty acids produced has an impact on the biodiesel quality. According to Stansell *et al.* (2012), a large proportion of saturated and mono-insaturated and a small proportion of poly-insaturated fatty acids in the oil would meet American and European biodiesel standard.

Therefore, the purpose of this study was first to measure the productivity gained when growing algae under mixotrophic conditions and to compare it with phototrophic conditions. Then, it aims to assess the potential of CH and DBP to replace glucose as organic carbon source for this mixotrophic culture. For this, we compare the effects on growth kinetics, on biomass productivity, and on neutral lipids content in cells of the studied algae-bacteria consortium. In order to compare the stability of the strain under testing conditions, its integrity was measured by using an index based on the proportion of the number of *Chlorella sp.* on the total number of observed microorganisms. Finally, fatty acids produced by the strain were analyzed to guide the choice of the fuel to be produced and to evaluate whether phototrophy or one of the tested organic carbon sources influenced their proportion in the cells.

Materials and methods

Strain and culture

The algae-bacteria consortium cultivated in this study is a native strain, mainly composed of *Chlorella sp.,* which was isolated in previous works from the aluminum smelter effluents. The seed culture has been cultivated in aluminum smelter effluent based medium, to which Bold Basal medium nutrients were added (Bischoff and Bold 1963) with the following modifications: KNO_3 1.443 g/L, KH_2PO_4 0.877 g/L, $MgSO_4 \cdot 7H_2O$ 0.3 g/L, ferric sodium EDTA 0.028 g/L. Organic carbon was also added by fed-batch for a total concentration of 1 g/L of glucose per culture cycle.

Composition of alternative sources of organic carbon

The organic carbon is present mainly in the form of reducing sugar (glucose and xylose) in CH and in the form of lactose in DBP. In both compounds, the nitrogen is primarily in the form of protein. The concentrations of total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) were analyzed (Table 1) to adjust them by dilution at the same level for each treatment.

Experiments

The inoculum used for this study was collected from exponential growing seed culture. Its concentration was 4.75 x 10^7 cells mL^{-1} and its volume was 10% (v/v) of the medium. Cultures were performed in 1L Erlenmeyer flasks containing 500 mL of medium. They were shaken on orbital shaker at a speed of 130 rpm, under fluorescent lighting 12 h / day with an intensity of 20 µmol photons$\cdot m^{-2} \cdot sec^{-1}$ (measured by a photometer Model LI-250 of LI COR, Lincoln, USA).

The temperature was controlled at 21°C and the pH was measured on a daily basis (pH meter Symphony, SB70P model VWR, Radnor, USA). Four treatments were tested in triplicate: phototrophy (without organic carbon), mixotrophy / glucose, mixotrophy / DBP and mixotrophy / CH. For mixotrophic treatments, an equivalent amount of TOC was added to the flasks through the dilution of carbon sources. The culture medium of all treatments is composed of the smelter's wastewaters enriched with the nutrients mentioned above. The amount of added KNO_3 was adjusted in function of TKN content in carbon sources.

The duration of the culture was 9 days. This cycle included a 4-day acclimation period used to enhance the consortium stability before the addition of organic carbon. Then, an effective period of 5 days started at the time of the first organic carbon injection. It was added by fed-batch through 2 injections of 1.03 g/L one day interval. This concentration, as well as that of other nutrients, were determined during previous optimization experiments.

Analyses

Biomass

Biomass at the end of culture was quantified according to the method of Borowitzka and Moheimani (2013) modified. A sample of 10 ml was filtered through preweighed filters (glass microfiber 934-AH from Whatman, porosity 1.5 µm). After rinsing with demineralized water, the filters were dried in an oven at 60°C for 24 hours and were weighted. Biomass productivity was calculated for the exponential growth phase period according to the following equation (1):

$$(1)\ P_b = \left(B_f - B_i\right) / T$$

Where:

P_b = Biomass productivity ($g \cdot L^{-1} \cdot j^{-1}$)

B_f = Final biomass ($g \cdot L^{-1}$)

B_i = Initial biomass of the effective period of culture ($g \cdot L^{-1}$)

T = Number of days of the effective period

Neutral lipids

Neutral lipids were quantified by gravimetry after their extraction from the harvested biomass at the end of culture, according to the method of De la Hoz Siegler Jr (2011) modified. Biomass has previously been dried after rinsing with demineralised water, by a drying apparatus under vacuum (Savant Speedvac Systems, SS21 model Thermo Savant, Thermo Fisher Scientific Inc. Waltham, USA). A dried biomass of 0.05 g weight per sample, in duplicate, was ground in liquid nitrogen and soaked overnight in 5 ml of hexane. Biomass and solvent were then exposed to ultrasound (20 hrtz) with a device (Sonifier Cell Disruptor Model 350 from Branson Sonic Power Co, Danbury, USA) fitted with a microprobe (model A3-561 from Branson Ultrasonics Corporation, Danbury, USA) to break all cells. Cell debris were separated from the liquid phase by centrifugation (5000 G, 10 min). The solvent was evaporated and the oil

was weighed. The amount of accumulated lipids in cells is expressed as a percentage of the dried weight of the biomass (% w /w).

Integrity of the consortium

A counting of algal cells was also carried out daily with a Neubauer counting chamber by using a phase contrast microscope (Axio Scope A1 model from Zeiss, Oberkochen, Germany) according to the method described by Guillard and Sieracki (2005). Indeed, we added the counting of microorganisms considered as biological contaminants in the consortium: algae other than *Chlorella sp.*, protozoa, rotifers and fungus. As hyphae cannot be counted individually, their number was estimated by converting their covering surface of the Neubauer chamber into a number of algae according to an average area of 20 μm^2. These countings were then used to calculate the index of integrity of the consortium, whose maximum is 100, according to the following equation (2):

$$(2)\quad I=100-\sum(C_i / A_i \times 100) / T$$

Where:

I = Integrity (%)

C_i = Total biological contaminants

A_i = Total consortium algae in the Neubauer chamber

T = Number of days of effective period

i = Day of culture

Lipid profile

The fatty acid composition of the lipids extracted was analyzed by GCMS after a methyl transesterification according to the method of Li *et al.* (2013). 2.5 mL of a solution of BF 3 in methanol (14% v/v) were added to extracted lipids and heated at 65°C for 20 min in saddled tubes. One milliliter of saturated NaCl solution and 1 ml of hexane were then added. After being agitated, the tubes were centrifuged and the

supernatant was collected to analyze the fatty acid methyl esters (FAME) with a gaseous chromatograph coupled to a mass spectrometer (Agilent 7820A model, Santa Clara, USA) having a capillary column DB-WAX (30 m, 0.25 mm d.i., 0.25 mm film thickness) connected to a mass spectrometer Agilent 5977E. The injection temperature was 90°C. Helium was used as a carrier gas (10.5 psi, 90°C). The mass spectra were recorded under electron ionization (70 eV) at a frequency of 2.5 times per second in a range of 50-650 m/z. The initial temperature of 100°C was increased 2 minutes after the injection of 10°C min^{-1} until 140°C. The temperature was then raised to 250°C at intervals of 5°C min^{-1}. The identification and quantification of FAME were performed by using the mass spectra standards Supelco®37 Component FAME Mix (Bellafonte, USA).

Statistics

Statistical analyzes were performed by using JMP software 10.0 SAS Institute Inc. (NC, USA). The results of biomass productivity, neutral lipids content in cells and the integrity of each replica were analyzed by a one-way ANOVA followed by a HSD Tukey-Kramer test (α 0.05). Fatty acids were analyzed by linear regression (least squares method standard).

Results

The algae-bacteria consortium dominated by *Chlorella sp.* was cultivated in mixotrophy with two alternating organic carbon sources to assess the potential of these sources to replace glucose for biofuel production in industrial wastewaters. Mixotrophic cultures were also compared to a phototrophic culture to determine whether the addition of organic carbon stimulated productivity. The maximum biomass was reached with glucose (1.42 ± 0.05 g·L^{-1}), followed by DBP (1.15 ± 0.11 g·L^{-1}), CH (0.90 ± 0.08 g·L^{-1}) and the control under phototrophy (0.62 ± 0.05 g·L^{-1}) (Fig. 1). The growth kinetics of the strain shows that the exponential phase ends at day 7 in all treatments. However, a slowdown occurs after the day 6 in the glucose treatment. Although glucose made it

possible to reach a higher biomass, no significant difference in the consortium productivity between various organic carbon sources was detected. Since the gain in biomass in all treatments was weak or even negative after 7 days, the productivity was calculated according to the number of days since the beginning of the effective phase to the end of the exponential growth phase (5 days 7) (Fig. 2). In descending order, it was 0.47 ± 0.04 g·L^{-1}·j^{-1}, 0.45 ± 0.15 g·L^{-1}·j^{-1} and 0.32 ± 0.06 g·L^{-1}·j^{1} for DBP, glucose and CH, respectively. Only the control under phototrophy has a significant lower productivity compare to DBP and glucose ($p<0.05$) with 0.17 ± 0.01 g·L^{-1}·j-1. Therefore, the addition of organic carbon increases productivity from 2.6 to 2.8 times, according to the source.

However, the cellular neutral lipids content was significantly higher in the control treatment ($p < 0.05$), with $3.0 \pm 0.4\%$, (Fig. 2) although the difference was small. No significant differences in lipids have been detected between carbon sources. The percentage of neutral lipids was 2.0 ± 0.4 for glucose, 1.8 ± 0.2 for DBP and 1.6 ± 0.2 for CH.

To maintain a stable productivity in open tank, controlling contamination from undesirable microorganisms has a particular importance. The development of an integrity index aimed at comparing the level of strain stability under mixotrophy and phototrophy modes, as well as according to the selected organic carbon sources. The consortium integrity index remains very high in all treatments (Table 2). It is however significantly lower with CH, even if the difference is very small.

In addition to productivity and stability of the culture, algae lipids profile is a parameter to be considered in order to define the most appropriate valuing path for algal biomass. Fatty acids produced by the strain were analyzed to determine if phototrophy or one of tested organic carbon sources influenced their proportion. The major FAMEs detected are C16:0 (16.2 to 32.4%), C18:2 (12.2 to 20.7%) and C18:3 (n-3) (7.5 to 28.4%). Only FAMEs showing a proportion greater than 1% of the profile in more than one treatment are presented in Table 3.

Linear regressions shown that the proportions of C12:0, C14:0 and C18:3 (n-3) were influenced by a categorical variable defined by the presence and the nature of organic carbon sources. Despite that part of the fatty acids variation thereof is not explained by this variable, coefficients of determination (R^2) of the models show that it has a significant influence to α threshold equal or less than 0.05 (table 4). These three FAMEs vary similarly in each treatment (Fig. 3). Their proportion is depending on model, top for CH, followed by phototrophy, DBP and glucose.

Discussion

Microalgae which grown under phototrophic conditions accumulate a larger proportion of lipids (% w/w) at the end of culture but the lipids productivity ($g \cdot L^{-1} \cdot j^{-1}$) was greater in mixotrophic treatments since they have a higher biomass production.

Despite glucose has been demonstrated to be easier assimilated than other organic carbon sources (Gao *et al.* 2010), no significant differences were detected in the biomass productivity by ANOVA analysis between organic carbon sources. DBP contains some organic carbon forms more complex than glucose (eg. lactose), beside monosaccharides (eg. galactose) and organic acids (eg. lactic acid), such as in the lactoserum (González *et al.* 1997). Sun *et al.* (2008) studied the growth of the strain *Chlorella zofingiensis* ATCC 30412 under heterotrophic conditions with galactose, lactose or glucose. Results showed that galactose was assimilated, but less efficiently than glucose, and lactose was poorly metabolized considering the residual amount in the medium at the end of culture. Davies *et al.* (1994) reported that an activity of β-galactosidase enzyme was detected in *Chlorella sp.* This enzyme catalyzes the breakdown of lactose into glucose and galactose. Bacteria present in the consortium could also have played a role in the degradation of complex sugars, while having a positive influence on the growth of algae. For example, acetic acid is a metabolite produced by certain bacteria (Raspor and Goranovic 2008). In our condition (pH 7-8, data not shown), acetic acid was in the form of acetate. Some *Chlorella sp.* have the ability to assimilate acetate as their biomass productivity increases when it is present (Heredia-Arroyo *et al.* 2011; Rai *et al.* 2013).

However, further research should be conducted to understand microbial ecology of the consortium.

DBP also contains organic nitrogen, especially in the form inter alia of protein. This form of nitrogen, although more complex, seems to be preferred by certain strains of microalgae rather than mineral nitrogen (Gao *et al.* 2010; Xiong *et al.* 2008). Furthermore, the DBP-containing milk residues, can contain some of the vitamins found in milk (FAO 2014). Among them, vitamin of B_{12} complex are essential to the growth of several species of algae (Barsanti and Gualtieri 2014) and an increase in their concentrations in the medium can increase biomass productivity (Croft *et al.* 2005). These authors also highlighted the acquisition vitamin B_{12} phenomenon by algae through symbiotic associations with bacteria.

Regarding cellulosic hydrolysate, glucose and xylose forms are in the majority of organic carbon. Most studies that have been tested xylose as sugar source in heterotrophic/mixotrophic culture reported that it did not or little contribute to microalgae's growth, no matter the strain (Kim *et al.* 2012; Rodríguez-López 1966; Samejima and Myers 1958). However, recent studies demonstrate the ability of *Scenedesmus obliquus* and *Chlorella sorokiniana* strains to use xylose as a carbon source in mixotrophy (Yang *et al.* 2014; Zheng 2013). The xylose assimilation would be otherwise inhibited in the presence of glucose (Zheng 2013) due to a higher affinity of the glucose with the membrane transporter. The consortium could therefore have assimilated xylose after consuming glucose in the culture medium. On the other hand, studies on the use of enzymatic hydrolysates of complex compounds as organic carbon source demonstrate their effectiveness. For example, Gao *et al.* (2010) have experienced enzymatic hydrolysate of sorghum syrup, an African plant rich in sucrose, glucose and fructose. Growth of *Chlorella protothecoides* strain was higher with this hydrolysate compared to glucose. Xu *et al.* (2006) obtained similar results with enzymatic hydrolysate of corn powder.

The presence of organic nitrogen in the CH, although in lower concentration than in the DBP, may also have stimulated the growth of algae. Previous experiments (data not shown) made with CH and containing a lower concentration of mineral nitrogen (KNO_3) have also allowed the strain to achieve a productivity higher than that of the present study, nearly surpassing glucose. This suggests that the optimum nitrogen concentration may vary from an organic carbon source to another. It would then be necessary to optimize the concentration of nitrogen for a specific carbon source.

The addition of organic carbon affects only slightly the consortium integrity index during the growing period. The cultivation mode thus favors the stability of the consortium despite that the risks of contamination is increased by the presence of organic carbon. The CH treatment integrity index is significantly lower, but the difference is probably too small to have had a significant impact on productivity. Thus, this study suggests that the addition of alternative organic carbon sources such as CH and DBP does not disturb the integrity of the consortium and does not favor the emergence of undesirable microorganisms.

In terms of lipids, although their concentrations ($g\ L^{-1}$) in DBP and CH culture are equivalent to those with glucose, the produced amount is low in all treatments. The strain used for this study was selected for their stability in the wastewater based medium. This is the most important characteristic to succeed a mixotrophic cultivation in open tank. However, this choice can be done at the expense of lipid productivity. Moreover, the majority of fatty acids produced by the consortium are saturated (35% to 52%) and polyunsaturated (28% to 52%). According to Ramos *et al.* (2009), the oil produced by the consortium have combustion properties comparable to conventional diesel. However, the presence of a large proportion of polyunsaturated fatty acids could affect the oxidative stability while the high concentration of saturated fatty acids could interfere with the fluidity of low temperature fuel. The obtained results show that the organic carbon source can influence the concentration of certain fatty acids in the consortium (C12:0, C14:0 and C18: 3n3). Despite this, the quality of biodiesel could not be improved, because their proportion variation is too low to have a significant impact

on fuel properties. For these reasons, a conversion of biomass into crude oil by hydrothermal liquefaction rather than biodiesel could be more advantageous. Hydrothermal liquefaction increases the oil yield by converting non-lipid molecules into oily compounds (Biller and Ross 2011). Thus, the produced biocrude could potentially replace fuel oil in process (Peterson *et al.* 2008).

Conclusion

To conclude, the alternative organic carbon sources DBP and CH could substitute glucose for mixotrophic production of the studied algae-bacteria consortium, without affecting the consortium integrity. However, further experiments are needed to assess the long-term stability of the consortium and in greater volume. The use of DBP and CH could help reduce fuel production costs and foster the process profitability. Both organic carbon sources are less expensive than pure glucose and can reduce the nutrient cost of mixotrophic production of microalgae. But, the neutral lipids extraction from strain for their conversion into biodiesel would not be a viable choice because of its low content and its unfavorable lipid profile. Hydrothermal liquefaction could be a more advantageous conversion mode since it would use the entire biomass for a potentially higher yield oil.

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Tables

Table 1: Total organic carbon (TOC) and total Kjeldahl nitrogen (TKN) concentrations of alternative sources of organic carbon

Alternative sources	TOC (g/L)	TKN (g/L)
CH	28.68	0.50
DBP	21.69	1.11

Table 2: Integrity index of algae-bacteria consortium under different culture conditions

Conditions	Integrity index (SD)
Phototrophic	99.8 (0.08)
Gluc	99.9 (0.04)
DBP	99.3 (0.02)
CH	98.3 (0.6)

Table 3: Fatty acids methyl esters (FAMEs) present in a proportion greater than 1% of the profile in more than a treatment (standard deviation)

Fatty acids	Phototrophic	Gluc	DBP	CH
C10:0_Decanoic	7.7 (4.5)	2.2 (0.5)	9.5 (3.4)	3.5 (1.9)
C12:0_Dodecanoic	5.5 (3.0)	1.9 (0.4)	8.4 (2.9)	3.4 (1.8)
C14:0_Methyl tetradecanoate	3.9 (2.5)	1.7 (0.6)	8.5 (3.7)	3.6 (1.9)
C14:1_Methyl myristoleate	5.8 (6.8)	3.1 (4.7)	4.7 (3.8)	6.3 (6.3)
C16:0_Hexadecanoic	**23.2 (12.3)**	**23.8 (7.6)**	**16.2 (2.2)**	**32.4 (22.5)**
C16:1_9-Hexadecenoic	0.9 (0.7)	0.7 (0.2)	4.2 (3.1)	0.7 (0.2)
C17:1_Cis-10 Heptadecenoic	2.8 (2.1)	5.9 (2.0)	1.9 (1.5)	3.5 (0.9)
C18:0_Mehtyl stearate	5.1 (1.2)	2.5 (0.8)	3.7 (1.3)	3.1 (1.4)
C18:1 (n-9)_9-Octadecenoic	0.9 (0.6)	1.7 (0.9)	4.8 (4.2)	2.7 (2.0)
C18:2_9.12-Octadecadienoic	**15.0 (13.4)**	**20.7 (2.9)**	**12.2 (6.6)**	**15.8 (13.4)**
C18:2 (n-6)_9.12 -Octadecadienoic	1.5 (0.6)	1.0 (1.4)	0.7 (0.3)	0.3 (0.2)
C18:3 (n-6)_Gamma-Linolenic	1.4 (0.2)	0.4 (0.0)	6.1 (2.4)	0.8 (1.0)
C18:3 (n-3)_9.12.15- Octadecatrienoic	**17.3 (15.5)**	**28.4 (9.8)**	**7.5 (3.7)**	**19.1 (18.2)**
C20:3_8.11.14-eicosatrienoic	0.6 (0.6)	1.2 (0.4)	1.4 (1.2)	0.1 (0.1)
C20:4 (n-6)_5.8.11.14- Eicosatetraenoic	0.9 (1.1)	0.7 (1.2)	0 (0)	0.5 (0.6)

Table 4: Adjustment and ANOVA of linear models for fatty acids varying according to presence and source of organic carbon

	Adjustement		ANOVA					
Fatty acids	**R^2**	**R^2 ajust**	**Source**	**D.f[a]**	**Sum of squares**	**Mean square**	**F ratio**	**p-value**
C12:0	0.63	0.46	**Model**	3	72.0459	24.0153	4.6041	0.04
			Residual	8	41.7284	5.2161		
			Total	11	113.7743			
C14:0	0.61	0.46	**Model**	3	74.6397	24.8799	4.1598	0.05
			Residual	8	47.8478	5.9810		
			Total	11	122.4875			
C18:3 (n-3)	0.83	0.76	**Model**	3	63.5821	21.1940	12.7820	0.002
			Residual	8	13.2650	1.6581		
			Total	11	76.8471			

Captions and figures

Captions

Fig. 1

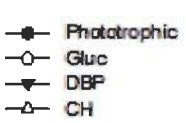


Fig. 2

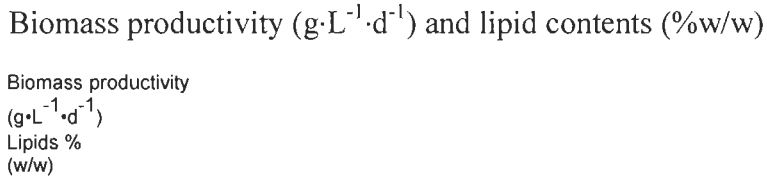
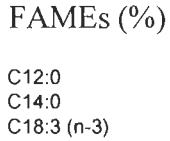


Fig. 3



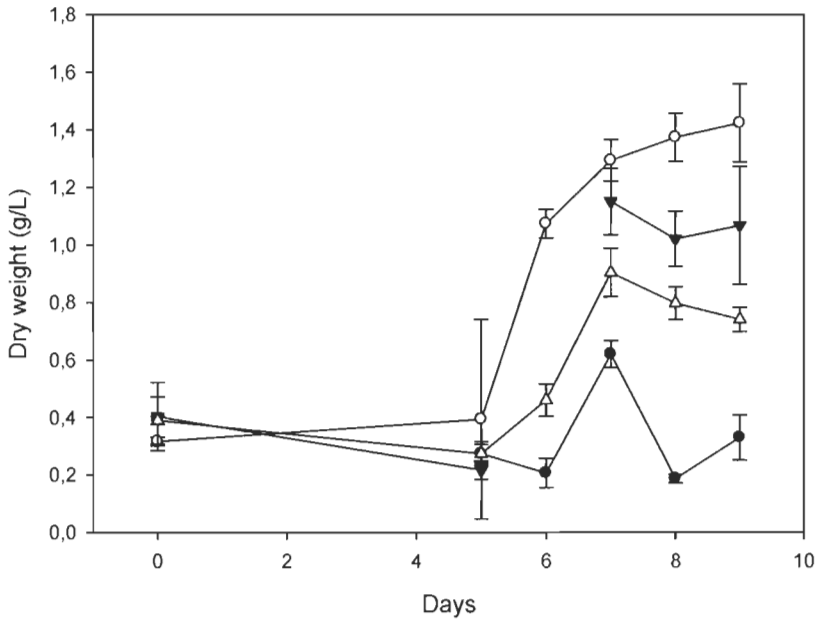


Figure 1: Growth kinetics of algae-bacteria consortium (Gluc = Glucose, DBP = Dairy byproduct, CH = Cellulosic sugar).

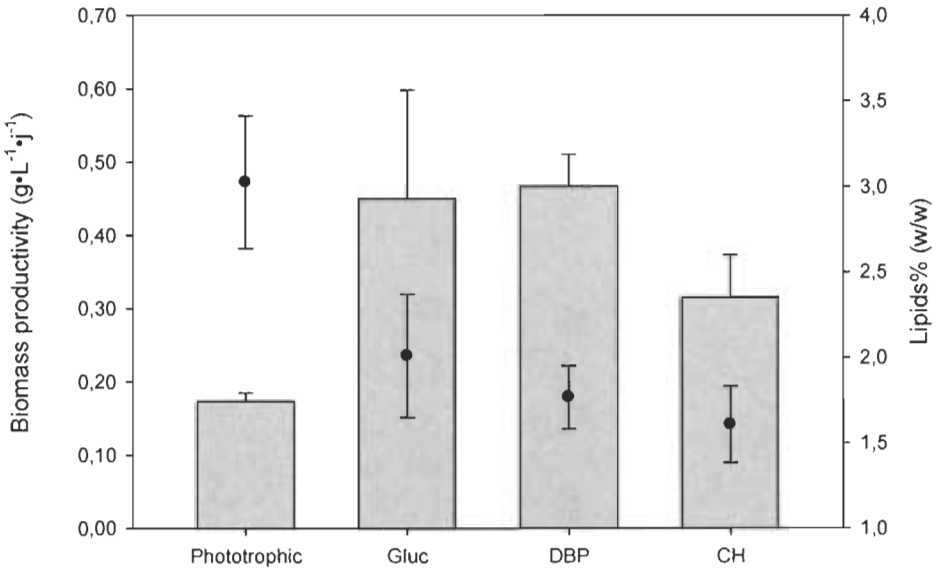


Figure 2: Biomass productivity and cells proportion of neutral lipids according to organic carbon source (Gluc = Glucose, DBP = Dairy byproduct, CH = Cellulosic sugar).

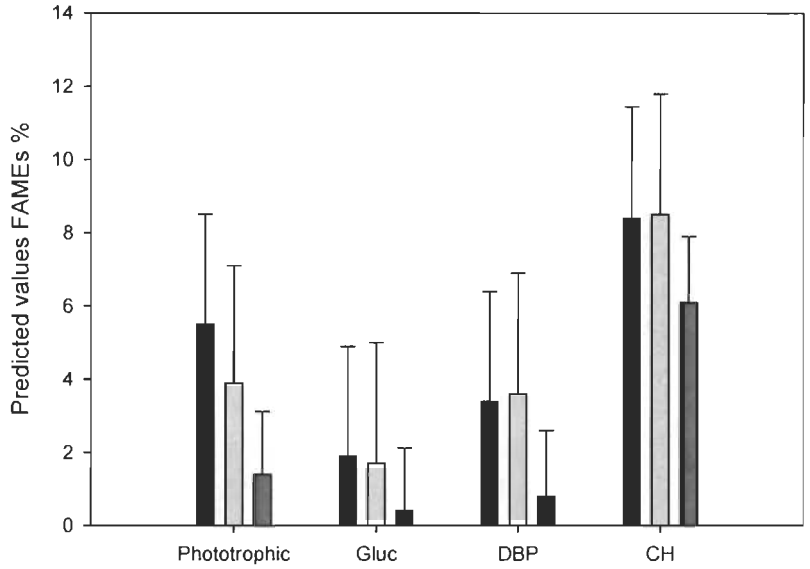


Figure 3: Predicted values of the models on the proportion of FAMEs C12:0, C14:0 and C18:3 (n-3) as a function of the presence and the organic carbon source.

CHAPITRE IV

CONCLUSION

La production de biomasse algale dans des eaux usées à des fins énergétiques fait actuellement l'objet de recherches très actives dans le domaine de la bioénergie. Les microalgues offrent une solution de remplacement aux énergies fossiles tout en procurant des bienfaits environnementaux. Des défis économiques restent à surmonter pour l'application commerciale et à grande échelle de tels procédés. La cohabitation d'une production de microalgues avec une industrie existante permettrait d'en relever certains.

La présente étude avait pour but d'élaborer un milieu de culture à base des eaux usées de l'usine Rio Tinto Alcan située à Alma (Québec). Contenant trop peu de nutriments, ces eaux doivent être enrichies par des apports externes, en proportion favorable à la productivité des microalgues et par des composés peu coûteux pour assurer la rentabilité du projet. Le besoin en nutriments est spécifique à chaque souche de microalgues et peut varier selon les conditions de cultures. Celles du présent projet étant particulières et peu documentées, il importait donc d'étudier cet aspect. L'apport en carbone organique constitue un coût majeur dans la production de microalgues. Cet aspect devait ainsi être intégré dans l'évaluation technico-économique du projet dans son ensemble. Finalement, l'évaluation du profil des acides gras était essentielle pour orienter le choix de la valorisation énergétique de la biomasse algale la plus appropriée parmi tous ceux possibles (combustion, biodiesel, biogaz, huile brute, etc.).

Les travaux présentés dans le premier article de ce mémoire portaient sur l'objectif 1 et 3 (chapitre II). En ce qui concerne les concentrations en nutriments (C, N, P), le carbone organique n'a pas eu d'effet significatif sur la productivité en biomasse et en lipides, dans les concentrations testées. L'azote en concentration maximale (0,200 g/L) et le phosphore en concentration minimale (0,003 g/L) procuraient la

productivité la plus élevée du consortium CHRT-A, soit 0,93 $g \cdot L^{-1} \cdot j^{-1}$ et 0,023 $g \cdot L^{-1} \cdot j^{-1}$ selon les modèles de la biomasse et des lipides. Le plan d'expérience utilisé, un factoriel 2^3, évaluait les relations linéaires entre les facteurs et les variables réponses puisqu'il comportait deux niveaux, en excluant les points centraux. Afin de compléter les résultats de ce design, une augmentation de plan par l'ajout d'un 3^e niveau pourrait être réalisée. L'effet du carbone organique sur la productivité pourrait possiblement être décelé dans le modèle quadratique généré par ce design augmenté. Par la même occasion, des concentrations plus élevées en carbone organique pourraient être testées. Bien que plusieurs études ont été réalisées sur l'effet de la concentration en nutriments sur la croissance de souches de microalgues, peu d'entre elles ont utilisé un design expérimental. Cette approche expérimentale a permis d'étudier les interactions entre ceux-ci par le croisement de nombreuses conditions.

Au niveau de la qualité de l'huile extraite lors des travaux de l'objectif 1, les principaux acides gras détectés étaient les acides palmitique (C16:0), stéarique (C18:0), et oléique-élaïdique (C18:1 n-9). Les modèles ont démontré que les concentrations en nutriments influençaient la proportion de certains acides gras dans un écart maximal de 12 % ($p<0,05$). Selon les informations tirées de la littérature, cet écart ne serait toutefois pas assez important pour avoir une influence sur les propriétés globale de l'huile, compte tenu de leur proportion minoritaire. Cette étude a permis de définir la limite d'action des nutriments sur le profil lipidique de la souche CHRT-A. Rares sont les études qui ont abordé l'effet de la concentration en plusieurs nutriments sur la proportion des acides gras accumulés par un design expérimental qui, encore une fois, permet de croiser plusieurs conditions différentes.

Malgré que la concentration optimale en nutriments soit spécifique à chaque souche et qu'une autre souche a été utilisée par la suite, les résultats obtenus à l'objectif 1 ont fourni des informations essentielles pour la poursuite du projet RTA. D'abord, il est possible d'atteindre la productivité en biomasse visée dans le projet (0,4 g/l/j) avec un consortium algues-bactéries cultivé dans les eaux usées de l'aluminerie enrichie avec des nutriments de base (C, N, P, Mg et Fe). De plus, avec une

souche de microalgues contenant une faible proportion de lipides cellulaires, il convient de fournir les concentrations en nutriments favorisant la productivité en biomasse au détriment de l'accumulation cellulaire de lipides. En effet, il a été démontré que la productivité en biomasse influence davantage l'augmentation de la productivité en lipide. Finalement, les concentrations en nutriments peuvent influencer la proportion de certains acides gras, mais dans un ordre de grandeur limité. La manipulation du profil lipidique pour orienter l'huile vers les propriétés désirées ne peut donc pas reposer seulement sur la variation des concentrations en nutriments.

L'étude de résidus agroalimentaires comme source alternative de carbone organique, qui constituait l'objectif 2, a été présentée dans le 2^{e} article de ce mémoire (chapitre III). Ces travaux ont été réalisés avec la souche CHRT-B. Bien qu'aucun modèle statistique sur les facteurs C, N et P n'a pu être réalisé au préalable, des expériences préliminaires avaient permis de déceler des tendances et les concentrations en ces nutriments ont été fixées en conséquence pour les expériences sur les sources alternatives de carbone organique. Les deux résidus testés, l'hydrolysat cellulosique et le sous-produit laitier ont permis au consortium algues-bactéries d'atteindre une productivité en biomasse et une proportion cellulaire de lipides similaire à celle obtenue avec le glucose. La productivité en biomasse a été de 0,47 ± 0,04 $g \cdot L^{-1} \cdot j^{-1}$ avec le SPL, de 0,45 ± 0,15 $g \cdot L^{-1} \cdot j^{-1}$ avec le glucose, de 0,32 ± 0,06 $g \cdot L^{-1} \cdot j^{-1}$ avec le HC et de 0,17 ± 0,01 $g \cdot L^{-1} \cdot j^{-1}$ avec le contrôle en phototrophie. L'accumulation de lipides neutres a été de 3,0 ± 0,4 % pour le contrôle en phototrophie, 2,0 ± 0,4 % pour le glucose, 1,8 ± 0,2 % pour le SPL et 1,6 ± 0,2 % pour le HC. De plus, cette étude a démontré l'avantage d'une culture mixotrophique par rapport à une culture phototrophique par le gain de productivité en biomasse de plus de 240 % (pour une intensité lumineuse de 20 µmoles m^{-2} s^{-1}). Les concentrations en azote et phosphore devront cependant être optimisées pour chacun de ces composés. Afin de confirmer le potentiel de ces composés, le suivi d'une culture à plus long terme et en plus grand volume devra également être réalisé. La littérature comporte plusieurs études sur des sources alternatives de carbone utilisées pour la croissance de microalgues. Beaucoup d'entre elles concernent des composés simples (ex. glycerol, acetate, lactate) et certaines ont

testé des composés plus complexes (ex. mélasses, drêche, lactosérum). La plupart ont par ailleurs été réalisées dans des milieux standards et stériles. La présente étude a approfondi davantage le contexte d'application industrielle, en testant ces sources alternatives dans des eaux usées en conditions non stériles.

Concernant l'objectif 3, le 2^{e} article aborde l'effet des sources de carbone sur le profil des acides gras de la souche CHRT-B (chapitre III). Comme pour les concentrations en carbone azote et phosphore, malgré leur influence sur quelques acides gras, la variation reste mineure et n'affecterait donc pas les propriétés du biodiesel. De même, l'extraction des lipides neutres de la souche pour leur conversion en biodiesel ne serait pas un choix rentable en raison de sa faible teneur en lipides et de son profil lipidique non favorable. La liquéfaction hydrothermale pourrait constituer un mode de conversion plus avantageux puisqu'elle permettrait d'utiliser la totalité de la biomasse pour obtenir un rendement en huile possiblement plus élevé.

En somme, l'hydrolysat cellulosique et le sous-produit laitier pourraient potentiellement remplacer le glucose dans le cadre du projet pour une culture mixotrophe à grande échelle de consortium algues-bactérie (CHRT-B) dans les eaux usées d'une aluminerie. Les résultats de cette étude seront pris en compte dans les calculs technicoéconomiques du projet RTA et orienteront certaines décisions stratégiques.

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