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MODELAGEM DE CRESCIMENTO CELULAR E CONSUMO DE DIÓXIDO DE CARBONO POR CIANOBACTÉRIAS CULTIVADAS EM FOTOBIORREATORES

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"Um dia, a Terra vai adoecer. Os pássaros cairão do céu, os mares vão escurecer e os peixes aparecerão mortos na correnteza dos rios. Quando esse dia chegar, os índios perderão o seu espírito. Mas vão recuperá-lo para ensinar ao homem branco a reverência pela sagrada terra. Aí, então, todas as raças vão se unir sob o símbolo do arco-íris para terminar com a destruição. Será o tempo dos Guerreiros do Arco-Íris."

Profecia feita há mais de 200 anos por "Olhos de Fogo", uma velha índia Cree.

Lema dos integrantens do Greenpeace (Rainbon Warrior)

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NOMENCLATURA

Abreviaturas		
BOP	Período De Operação Em Batelada	
FB	Batelada Total	
TOP	Período Total De Operação	
CCM	Mecanismo De Concentração De Carbono	
Mean obs	Média Dos Valores Observados	
Obs	Valores Observados	
Pred	Valores Preditos Pelos Modelos	
Siglas		
A	Ln (X_{max}/X_0)	-
A(t)	Função de ajuste	h
B	Crescimento Relativo no Tempo t	h^{-1}
C	Ln (X_0/X_0)	
C_C	Percentual De Carbono Na Biomassa	%
D	Taxa De Diluição	
K	Tempo necessário para atingir metade do $\mu_{máx}$	h^{-1}
K_I, K_{I2}	Constante De Inibição E Constante Auxiliar De Inibição	$mg.L^{-1}$
K_x	Constante De Saturação (Relativa À Biomassa)	$mg.L^{-1}$
n	Número De Amostragens Analisadas	-
M	Tempo Em Que A Taxa De Crescimento É Máxima	h
M_{CO2}, M_C	Peso Molecular Do CO_2, Peso Molecular Do C	g
P	Pressão Ambiente	atm
Q_g	Fluxo Volumétrico De Entrada De Gás	$L.h^{-1}$
q_{CO2}, q_{CO2_max}	Taxa De Fixação De CO_2; Máxima Taxa De Fixação De CO_2	h^{-1}
R	Constante Universal Dos Gases (0,082)	$atm.L.(mol.K)^{-1}$
S_0, S	Concentração Inicial Do Substrato E No Tempo t	$mg.L^{-1}$
T	Temperatura Do Meio De Cultivo	K
t	Tempo De Residência	h
V	Volume Reacional	L
X_0, X_{max}, X	Concentração Celular Inicial, Máxima E No Tempo t	$mg.L^{-1}$
Y	Ln (X_t/X_0)	-
$y_{CO2,e}$, $y_{CO2,s}$	Concentração De CO_2 Na Entrada E Saída De Gás	%
Símbolos Gregos		
λ	Duração Da Fase Lag	h^{-1}
μ	Velocidade Específica De Crescimento	h^{-1}
μ_{max}	Velocidade Máxima Específica De Crescimento	h^{-1}
ν	Curvatura Do Modelo	-

RESUMO

O aquecimento global é um dos mais sérios problemas ambientais da atualidade, gerando uma crescente preocupação com o meio ambiente. Dentre as possíveis formas de redução da concentraçao de dióxido de carbono, um dos causadores desse fenômeno, está a fixação biológica de CO_2 podendo ocorrer em fotobiorreatores alimentados com culturas de cianobactérias. Nesse sentido, através de uma colaboração com a refinaria de Paulínia, o estudo teve por objetivos avaliar o potencial dos efluentes líquidos da indústria petroquímica como meio de cultivo da cianobactéria *Aphanothece microscopica Nägeli* e determinar modelos de crescimento celular e consumo de dióxido de carbono. Os testes envolveram diferentes diluições do efluente líquido em água destilada (0, 25, 50, 75 e 100%) e diferentes suplementações de sais de BGN no efluente líquido (0, 25, 50, 75 e 100%), visando avaliar o crescimento celular do microorganismo. Os resultados demonstraram que a limitação de nutrientes no efluente afetava significativamente os resultados de aumento celular necessitando da adição de 25% sais. Esses dados também foram analisados em função de modelos de crescimento (Gompertz, Gompertz Modificado, Logístico, Morgan, Baranyi) sendo o melhor ajuste encontrado com o modelo Gompertz Modificado. Também foram simulados valores de produtividade em função de cultivo em regime contínuo revelando máxima produtividade aproximada de $1{,}41 kg_{biomassa}/L_{reator}$ em 1000h de operação com uma fixação de $2{,}61 kg_{CO2}/L_{reator}$. O consumo de dióxido de carbono foi avaliado em experimentos realizados apenas com meio BGN e tiveram como finalidade avaliar parâmetros como taxa máxima de consumo de CO_2, constante de saturação da biomassa e constante de inibição, sendo aplicados modelos de consumo de substrato (Webb, Aiva, Yano & Koga, Andrews, Chen e Ierusalinsky) para analisar os resultados e definir a condição de melhor concentração de biomassa para promover uma elevada fixação biológica de CO_2. O modelo de Andrews foi escolhido para representar esse processo de consumo de CO_2 pela facilidade no uso e significado físico de seus parâmetros indicando a possibilidade de se obter $3{,}00 g_{biomassa}.L^{-1}.h^{-1}$ e máxima taxa de retenção de CO_2 na forma de biomassa de $2{,}67 g_{CO2}.L^{-1}.h^{-1}$mostrando a potencialidade de uso desse sistema em processos de fixação biológica de dióxido de carbono.

Palavras Chave: microbiologia preditiva, CO_2, cianobactérias, biomassa, águas residuais.

ABSTRACT

Actually, global warming is one of the most serious environmental problems in the planet, because of it, the concerns with the environment have increased. Among the possible ways to reduce atmospheric carbon dioxide concentrations, one of the responsible for this phenomenon, is the biological fixation of CO_2, which can occur in photobioreactor feed with cyanobacteria cultures. Accordingly, through collaboration with Paulinia's refinery, this study had the objective to evaluate the petroleum industry wastewater as a culture medium to cyanobacteria *Aphanothece microscopica Nägeli* and to determine growth and CO_2 consumption models. The tests involved different dilutions of wastewater in ditilled water (0, 25, 50, 75 e 100%) e different supplementation of salts of BGN (0, 25, 50, 75 e 100%), to evaluate the development of the microorganism. The results showed that the nutrients limitation in the wastewater significantly affected cell growth requiring addition of 25% salts. These data were also analyzed in terms of growth models (Gompertz, modified Gompertz, Logistic, Morgan, Baranyi) and de better fit was found with the modified Gompertz model. Continuous cultivations was simulated and revealed a maximum productivity about of 1.41 $kg_{biomass}$ / $L_{reactor}$ at 1000h of operation with a setting of 2.61 $kg_{CO2}/L_{reactor}$. The consumption of carbon dioxide was evaluated in experiments realized with BGN and have the objective of measure parameters such as maximum rate of CO_2 consumption, biomass saturation constant and inhibition constant, been applied substrate consumption models (Webb, Aiva , Yano & Koga, Andrews, Chen and Ierusalinsky) to analyze the results and define the position of better concentration of biomass to promote a high biological fixation of CO_2. The model of Andrews was chosen to represent this process of consumption of CO_2 by the ease of use and physical meaning of its parameters indicating the possibility of obtaining 3.00 $g_{biomass}.L^{-1}.h^{-1}$ and the retention rate of 2.67 $g_{CO2}.L^{-1}.h^{-1}$ showing the potential use of this system in processes of biological fixation of carbon dioxide.

Keywords: Predictive microbiology, Carbon dioxide, Cyanobacteria, Biomass, Wastewater.

CAPÍTULO 1

Introdução geral

1 Introdução

O aquecimento global é um dos mais sérios problemas ambientais da atualidade, podendo gerar alterações em diversas áreas, incluindo distúrbios climáticos de precipitação de chuvas, derretimento das calotas polares ou mesmo a extinção de diversas espécies. Nos últimos anos, houve um crescente debate sobre a influencia do homem no aumento dos gases do efeito estufa (dióxido de carbono, vapor d'água, metano, óxido nitroso, hidrofluorcarbonos, perfluorcarbonos e hexafluoreto de enxofre). De acordo com diversos autores o dióxido de carbono é o principal gás causador desse fenômeno e contribui com aproximadamente 60% dos efeitos do aquecimento global, tendo sua concentração atmosférica aumentada de 285 ppmv no ano 1000 para 377 ppmv em 2004, com a estimativa de atingir 570 ppmv em 2100.

Nesse sentido, a crescente preocupação com o meio ambiente vem mobilizando vários segmentos do mercado, órgãos governamentais e indústrias para aplicar uma política ambiental que diminua os impactos negativos à natureza. Assim, o protocolo de Kyoto foi baseado no princípio da redução da emissão dos gases causadores do efeito estufa, especialmente na redução da quantidade de CO_2 liberado para a atmosfera. As formas de viabilizar essa redução podem ser divididas em três categorias: i) reduzir o gasto de energia, melhorando a eficiência de uso; ii) reduzir as emissões de CO_2 utilizando fontes de energia que não liberem esse gás e iii) desenvolver tecnologias limpas de captura e sequestro de CO_2. No caso das tecnologias limpas existem tentativas de tratamentos físicos, químicos e biológicos, onde a fixação biológica de CO_2, por culturas de cianobactérias em fotobiorreatores, atrai grande significado e importância devido à eficiência fotossintética desses microorganismos capazes de tolerar uma ampla gama de condições de luminosidade, salinidade, temperatura e pH.

Os fotobiorreatores utilizados nesse tipo de processo podem ser abertos ou fechados. A preferência por fotobiorreatores fechados começou a ser avaliada nos anos 90 em função da produtividade celular, menor perda de água por evaporação, diminuição da possibilidade de contaminação, dentre outras. Dentre as configurações mais usadas para fotobiorreatores fechados estão os do tipo coluna de bolhas e airlift. Para tornar esse processo mais viável economicamente tem sido estudada a possibilidade de tratamento biológico de efluentes líquidos associado ao tratamento de efluentes gasosos. Assim, a utilização de efluentes líquidos como meio de cultivo para as cianobactérias pode ser associada ao tratamento de gases (CO_2) sendo necessário destacar que o

levantamento cinético de fotobiorreatores em escala de bancada é função de cada espécie de cianobactéria e é a etapa inicial para o desenvolvimento desses processos em larga escala.

Apesar das inúmeras aplicações possíveis, apenas algumas espécies de algas e cianobactérias são cultivadas comercialmente devido ao pouco desenvolvimento das técnicas nos fotobiorreatores bem como pelo fato de que a escala comercial desta tecnologia ser fundamentada na viabilidade técnica e econômica da produção de compostos celulares de alto valor agregado. Assim é necessário controlar as variáveis operacionais, diminuir o risco de contaminação e minimização das perdas de água por evaporação. Atualmente são estudadas aplicações para essa biomassa que compreendem desde aplicações alimentícias e medicinais até geração de energia.

Neste sentido, a avaliação do potencial de uso de efluentes líquidos na produção de biomassa e a construção de modelos matemáticos e cinéticos são atividades essenciais para o desenvolvimento desses processos para maximizar a produção de biomassa e mitigação do dióxido de carbono e obter um processo isento de resíduos e tecnicamente viável.

2 Objetivo

2.1. Objetivo geral

Avaliar o desempenho de modelos de crescimento celular e de consumo de dióxido e carbono aplicados ao cultivo fotossintético da *Aphanothece microscopica Nägeli*.

2.2. Objetivos específicos

Avaliar a adaptação e crescimento da *Aphanothece microscopica Nägeli* quando cultivada utilizando o efluente líquido da refinaria de Paulínia;

Avaliar as taxas de crescimento celular, taxa de consumo de dióxido de carbono e produtividade;

Avaliar o desempenho de modelos de crescimento celular e consumo de dióxido de carbono no cultivo fotossintético da cianobactéria *Aphanothece microscopica Nägeli*;

Validar matematicamente e fenomenologicamente esses modelos;

Definir as condições operacionais apropriadas para o crescimento celular e fixação biológica de carbono;

3 Revisão Bibliográfica

3.1. Alterações Climáticas

O clima é um sistema complexo, constituído em função da interação entre atmosfera, superfície terrestre, neve, gelo, oceanos, massas de água e elementos vivos. É comumente definido em termos de média e variabilidade de temperatura, de precipitação e dos ventos, ao longo de um período de tempo (Australian Government – Bureau Of Meteorology, 2009). O clima varia em todas as escalas de tempo e espaço, ou seja, de um ano para o outro, bem como de uma década, século e milênio para o próximo, desde o local até o global (MacCracken & Perry, 2002) e evolui no tempo sob a influencia de sua própria dinâmica interna e, devido a mudanças nos fatores externos (Australian Government – Bureau of Meteorology, 2009). Essas mudanças são expressas em termos de forçantes radiativas, as quais compreendem a medida da influencia de um fator em alterar o balanço de energia de entrada ou saida do sistema atmosfera-terra e usadas para comparar como a contribuição humana e os fatores naturais influenciam no aquecimento ou no resfriamento do sistema climático (Ávila, 2007). Os Forçantes incluem erupções vulcânicas e variações solares, alterações na composição atmosférica influenciando o ciclo da água, ciclo de carbono, equilíbrio energético e diferentes características físico-químicas e biológicas dos processos que ocorrem dentro da atmosfera (Australian Government – Bureau of Meteorology, 2009). Sendo assim, a natureza complexa desta variabilidade é um grande obstáculo para a determinação das mudanças globais que são decorrentes das atividades humanas e daqueles que são função de fenômenos naturais.

A radiação solar é um dos fatores que mais afeta as alterações climáticas, sendo que de toda radiação solar que atinge a Terra, aproximadamente 340 $W.m^{-2}$, 30% é refletida e 70% é absorvida (pelas nuvens, gases estufa e superfície terrestre) e convertida em calor (Le Treut et al., 2007). Essa radiação é necessária para o aquecimento da superfície terrestre, contudo não é suficiente para a manutenção da vida, necessitando de um fenômeno natural chamado efeito estufa (Marion Koshland Science Museum of the National Academy of Sciences, citado por PEW Center on Global Climate Changes, 2009). Esse fenômeno faz com que a temperatura da Terra seja maior do que a que seria na ausência de atmosfera, sem ele a temperatura média da Terra seria -18ºC ao invés dos 15ºC que temos hoje, ou seja, 33ºC menor. Dessa forma, o sistema Terra-Atmosfera está em equilíbrio, pois toda a energia que entra é igual a que sai, mas seu saldo pode ser alterado por três formas fundamentais: i)

alterando a radiação solar recebida (mudanças na órbita da Terra ao Sol); ii) alteração da fração da radiação solar que é refletida (alterações na nebulosidade, partículas atmosféricas ou vegetação) e iii) alteração na radiação solar devolvida ao espaço (mudança de gases de efeito estufa aumentando a quantidade de radiação infravermelha que é absorvida antes de escapar para o espaço) (Le Treut et al., 2007; Australian Government – Bureau of Meteorology, 2009; INPE, 2009).

A concentração atmosférica de dióxido de carbono (CO_2), metano (CH_4) e óxido nitroso (N_2O) tem aumentado significativamente desde 1750 alterando o balanço de energia do planeta. Esse aumento da concentração atmosférica de dióxido de carbono se deve principalmente à queima de combustível fóssil e mudanças de uso da terra, enquanto o metano e o óxido nitroso são principalmente gerados pela agricultura (Ávila, 2007), Nesse sentido, nos últimos anos, houve um crescente debate sobre a influencia do homem no aumento da concentração atmosférica dos gases do efeito estufa (dióxido de carbono, metano, óxido nitroso, hidrofluorcarbonos, perfluorcarbonos e hexafluoreto de enxofre) (Shively et al., 2001), sendo geralmente aceito que, no século 20, o meio ambiente esteve sujeito a maior interferência humana de todos os tempos, podendo ser a causa do que atualmente é considerado um dos mais sérios problemas ambientais: Aquecimento Global (Wijanarko e Ohtaguchi, 2004; Yang et al., 2008).

O aquecimento global pode gerar alterações em diversas áreas, incluindo distúrbios climáticos, sendo que tanto a quantidade quanto a distribuição das chuvas e tempestades irão mudar radicalmente além de alterações nos padrões de ventos e eventos extremos do clima (secas, inundações, ciclones, tornados, tsunamis). Também ocorrerão mudanças na produção de alimentos, derretimento acelerado das calotas polares, aumento do nível dos oceanos, aceleração da extinção de algumas espécies da flora e fauna e muitos outros problemas ambientais de proporções distintas (IPCC, 2001; IPCC, 2007; Bilanovic et al., 2009). A magnitude e a velocidade com que essas mudanças climáticas irão ocorrer serão significativas, mas não completamente definidas (Reed & Assenz, 2009). Essas alterações teem sido continuamente monitoradas. Dados publicados pela NASA revelaram uma variação de 20% entre a extensão de gelo no oceano Ártico entre 1979 e o verão de 2005 (NASA citada por Pew Center on Global Climate Change, 2009).

O setor de energia seguido pelo setor industrial são os principais causadores das emissões dos gases do efeito estufa, participando com aproximadamente 26% e 19%, respectivamente, de toda emissão de dióxido de carbono ocorrida no ano de 2004 (IPCC, 2007). O principal gás do efeito estufa é o dióxido de carbono produzido tanto por fontes móveis (automóveis, dentre outros) quanto por fontes estacionarias (indústrias, dentre outras) e desbalanceando a distribuição de carbono na terra (litosfera, atmosfera e biosfera), sendo importante salientar que a quantidade total de carbono na terra é constante e esteve relativamente balanceada até antes da revolução industrial, onde as emissões de dióxido de carbono para a atmosfera aumentaram aproximadamente 31% de 1750 até 2001, com um ritmo de aproximadamente 0,4% por ano nas últimas duas décadas (Shively et al., 2001). Estudos da variação da concentração de CO_2 foram realizados através da análise de amostras de gelo coletadas na Antártica (determinação da concentração até 1900) e com coletores localizados no Hawaii (determinação de 1958 até 2004). Esses dados evidenciaram que entre 1000 e 1900 houve um aumento de 280 para 295 ppmv e de 315 para 377 ppmv de 1958 a 2004 (Yang et al., 2008). A concentração de metano na atmosfera também foi aumentada de aproximadamente 700 ppbv no período pré-industrial para 1721 ppbv em 1994 (Tomkiewicz, 2006).

De acordo com dados do IPCC (International Panel on Climate Change), a estimativa da concentração atmosférica de CO_2 em 2100 será superior a 570 ppmv, aumentando a temperatura terrestre entre 1,8 e 4,0ºC e elevando o nível do mar em 38m (IPCC, 2007) Nesse sentido, a crescente preocupação com aquecimento terrestre vem mobilizando órgãos governamentais e indústrias visando estabelecer políticas ambientais que reduzam os impactos negativos à natureza (Pelizer et al., 2007). O protocolo de Kyoto foi assinado em 15 de março de 1998 baseado no princípio da redução de 5,2% da emissão dos gases causadores do efeito estufa (em relação aos níveis de 1990), entre os anos de 2008 e 2012 (Protocolo de Kyoto, 1998). O foco é a diminuição da quantidade de CO_2 liberado para a atmosfera (Hsueh et al., 2009), existindo 3 possibilidades de se alcançar essa redução: i) reduzir o gasto de energia melhorando a eficiência do uso de energia; ii) reduzir as emissões de CO_2 usando fontes de energia que não necessitem da queima de combustíveis fósseis e iii) desenvolver tecnologias limpas de captura e sequestro de CO_2 (Yang et al., 2008).

Dentre as tecnologias de captura e sequestro de CO_2 existem tentativas de tratamentos físicos, químicos e biológicos. Dos vários desafios, a fixação biológica de CO_2, por culturas de cianobactérias

em fotobiorreatores, vem despertando elevado interesse por conta da promessa de ser uma tecnologia sustentável (Ono e Cuello, 2006; Yang et al., 2008; Araújo et al., 2008), pois se trata de uma estratégia onde o dióxido de carbono é incorporado à estrutura molecular das células na forma de proteínas, carboidratos e lipídios (Jacob-Lopes, 2007; Jacob-Lopes et al., 2008a).

As vantagens desse processo estão relacionadas à elevada eficiência fotossintética das cianobactérias quando comparadas às plantas superiores (Araújo et al., 2008). Isso ocorre devido ao fato delas possuírem numerosas características morfológicas (mecanismo de concentração de carbono, diferentes tipos de pigmentos para absorção luminosa em amplo espectro luminoso, tolerancia à elevadas concentrações salinas e de díoxido de carbono) que são resultantes dos fatores ambientais e que servem para maximizar o potencial fotossintético, conferindo grande flexibilidade e adaptabilidade à sobrevivência em condições extremas (Falkowski, 1997). Nesse sentido, é importante o estudo dessa tecnologia para a criação de processos sustentáveis de captura de dióxido de carbono bem como a geração de biomassa que pode ser transformada em fonte de energia ou mesmo utilizada em diversos ramos da indústria alimentícia e farmacêutica.

3.2. Cianobacterias e a *Aphanothece microscopica Nägeli*

Aproximadamente 2000 espécies de cianobactérias são conhecidas e podem ser divididas em 150 gêneros. São capazes de colonizar quase todos os habitats, desde oceanos até montanhas, de climas quentes à superfícies congeladas (Mundt et al., 2001; Sant'anna, 2006). Por causa dos distintos tipos de morfologia das cianobactérias e pelas características que são pouco conhecidas, a taxonomia desses microorganismos é constantemente redefinida (Pulz & Gross, 2004). Historicamente, as espécies de cianobactérias foram classificadas de acordo com o Código Internacional de Nomenclatura Botânica e com base em suas características morfológicas, anatomia, estrutura celular, fisiologia e metabolismo. Mais recentemente, elas tem sido classificadas por alguns bacteriologistas como cianobactérias ou cianófitas, sendo utilizado o Código Internacional de Nomenclatura de Bactérias (Anand, 1998; Meeting, 1996). Sua estrutura celular lembra bactérias gran negativas, mas normalmente elas vivem fotoautotroficamente (Mundt et al.m 2001), apresentam formas muito diversas, podendo ser arredondadas, cilíndricas, ovóides, estreladas e em forma de meia lua, e também apresentando vários níveis de organização, sendo unicelulares, coloniais ou filamentosas (Pádua, 2006; Radmer, 1996). São consideradas as precursoras dos cloroplastos das plantas superiores (Radmer, 1996).

Evidências fósseis sugerem que membros das famílias de cianobactérias *Oscillatoriaceae e Chroococcaceae* existiam há aproximadamente 3,5 Ga (bilhões de anos), sendo sua tolerância ecológica (sobrevivência em ampla gama de condições de luminosidade, salinidade, temperatura e pH bem como dessecamento prolongado e irradiação intensa) conseqüência dessa evolução precoce, ou seja, produto de seu grande sucesso em competir com outros micróbios precoces pelo espaço fotossintético disponível dessa época. Ao contrário da fotossíntese das cianobactérias (baseada em clorofila a que libera O_2), todas as outras formas de fotossíntese procariótica são anóxicas e baseadas em bacterioclorofila (inibida por O_2). Assim, as cianobactérias, com sua capacidade de produzir e tolerar O_2 teriam suplantado os fotoautótrofos anóxicos, sensíveis ao oxigênio, em grande parte da zona fótica do globo. Como resultado de seu êxito nesta competição por espaço fotossintético, as cianobactérias expandiram transformando-se, neste processo, em generalistas ecológicas excepcionais (Schopf, 1995) desenvolvendo papel central na evolução da biosfera atual, através da elevação do nível de oxigênio da atmosfera terrestre (Schmetterer, 1994; Jeon et al., 2005).

São organismos envolvidos na captura e utilização da luz solar por diferentes aparatos fotossintéticos e pigmentos (Collins et al., 2009), utilizando a luz solar como fonte de energia para crescimento e manutenção de seu metabolismo, possuindo potencial para elevada produtividade e sendo passíveis de cultivo em áreas instáveis para a agricultura (Morais et al., 2007). Estes organismos apresentam alta concentração de pigmentos, entre eles cita-se a clorofila *a* que é considerada essencial para a fotossíntese. Outras duas classes de pigmentos envolvidos na captação de energia luminosa são os carotenóides e as ficolibinas. Os carotenóides são pigmentos lipossolúveis de cor vermelha alaranjada ou amarela, encontrados em associação com a clorofila a nas cianobactérias. A terceira classe de pigmentos acessórios é a das ficolibinas, a ficocianina, pigmento azul presente nas microalgas e a ficoeritrina de cor vermelha, às vezes ausente (Metting, 1996). Além desses pigmentos esses microrganismos possuem um sistema intracitoplasmático altamente desenvolvido, indicando a fotossíntese como rota metabólica preferencial (Jacob-Lopes, 2007).

A fonte de carbono inorgânico para a fotossíntese desses microorganismos é o CO_2 livre e íons bicarbonatos que são transportados através da fina membrana plasmática e acumulados na célula como um reservatório de carbono inorgânico para a fotossíntese. O mecanismo de concentração de carbono (CCM – Carbon Concentration Mechanism) ocorre com diferentes estados de capacidade/afinidade

(maior capacidade do CCM em células que cresceram em baixas concentrações de CO_2 e menor nas que se desenvolvem em elevadas concentrações de CO_2) (Rodriguez-Buey e Orus, 2001). O CCM está dividido em 10 componentes básicos, como detalhado na Tabela 1.1 (Price et al., 2007).

Tabela 1-1: Componentes do CCM.

Componente	Atividade
1. Bombas de captação de C inorgânico	Pela necessidade de acumular ativamente C inorgânico para alcançar um nível satisfatório de fixação de dióxido de carbono. Foram identificadas 5 diferentes sistemas de captação: três de transporte de HCO_3^- e 2 de transporte de CO_2
2. Energia para captação de C inorgânico	A captação de C requer a entrada de energia metabólica. As formas de energia podem ser ATP, NADPH ou um gradiente eletroquímico de Na^+.
3. Acumulação de HCO_3^-	As células acumulam HCO_3^- independentemente da forma do carbono captado. Os valores internos das células podem chegar a 1000 vezes o valor da concentração externa
4. Manutenção de pH	Para que a célula não sofra os efeitos da constante variação de concentração de C inorgânico
5. Compartimento para a elevação e fixação de CO_2	Onde o HCO_3^- é usado para fornecer CO_2
6. Anidrase carbônica	Necessária para a conversão catalítica de HCO_3^- em CO_2
7. Barreira contra a perda de CO_2	Quando a concentração dentro da célula está elevada
8. Sistema de reciclo	Para o CO_2 que escapa do interior da célula
9. Regulação do CCM	Em função das concentrações externas de C inorgânico, ou seja, em função da disponibilidade de CO_2 e
10. Mecanismo de regulagem de captação de CO_2	Para evitar perdas de energia metabólica ou fuga de CO_2.

Experimentos sugerem que a indução da afinidade/capacidade do CCM deve ocorrer em duas etapas, a primeira promove uma rápida resposta à limitação de carbono e a segunda a completa adaptação que depende da síntese de alguns compostos. Alguns estudos também afirmam que perante a limitação de CO_2 ocorre um aumento da afinidade pelo bicarbonato. Acredita-se que o CCM nas cianobactérias envolve muitos processos e compostos (Rodriguez-Buey e Orus, 2001) e que as mesmas

são capazes de utilizar três diferentes vias de assimilação de carbono inorgânico: (1) assimilação direta do dióxido de carbono que passa pela membrana plasmática; (2) utilização de bicarbonato convertido em dióxido de carbono através da indução da enzima anidrase carbônica; (3) transporte de bicarbonato pela membrana plasmática (Grobbelaar, 2000).

No ambiente natural, assim como nos cultivos, o crescimento desses microorganismos é resultado da interação entre fatores biológicos, físicos e químicos (Raven, 1988). Os fatores biológicos estão relacionados às próprias taxas metabólicas da espécie cultivada, bem como com a possível influência de outros organismos sobre o desenvolvimento. Os fatores físico-químicos são estudados em função da influência da iluminação, pH, temperatura, salinidade e disponibilidade de nutrientes (Derner et al., 2006). O cultivo pode ser manipulado no sentido de induzir a produção de proteínas, ácidos graxos, vitamina A, pigmentos e outros bio-compostos bem como biomassa pode ser utilizada como suplemento alimentar na dieta humana ou de animais, incluindo a aquicultura (Morais et al., 2007).

A *Aphanothece microscopica Nägeli* pertence à família *choroococaceae*, formando colônias adaptadas à flutuação (Esteves,1988). Essas colônias são macroscópicas e amorfas, com mucilagem abundante, firme e rígida. Estruturalmente elas são elípticas acilíndricas (forma celular adulta), possuem conteúdo finamente granulado, medindo 9,0 - 9,5 μm x 4,2 μm, cerca de 2,1 vezes mais compridas que largas e a coloração é verde azulada escura (Halperin et al., 1974). Ela é encontrada nos corpos hídricos adjacentes à cidade do Rio Grande, RS, na forma unicelular e cujo aproveitamento poderá representar importante fonte de seqüestro de carbono.

Foram realizados estudos dessa cianobactéria envolvendo parâmetros ambientais, como os nutrientes nitrogenados e fosfatados (Stewart, et al., 1978; De Lorenzo, 1995), em relação à sua morfologia (Montoya e Golubic, 1991), em relação ao seu conteúdo lipídico (Queiroz et al., 2004), utilização na alimentação (Queiroz et al., 1999; Queiroz et al., 2000), remoção de CO_2 (Jacob-Lopes et al., 2008), produção de biomassa (Jacob-Lopez et al, 2008), efeitos dos ciclos de luz/escuro no crescimento celular (Jacob-Lopes et al., 2009) e diferentes respostas de crescimento, fotossíntese em diferentes condições de radiação UVB (Zeeshan e Prasad, 2009).

3.3. Fotobiorreatores

A utilização de fotobiorreatores com cianobactérias na biofixação de CO_2, requer a presença de luz, dióxido de carbono e nutrientes dissolvidos, ou seja, sistemas de iluminação, trocadores de

gases (adição de CO_2 e remoção de O_2), sistemas de adição de nutrientes e controle de temperatura (Rorrer e Cheney, 2004) e oferece vantagens pelo aumento da produtividade em condições controladas e com a relação área/volume otimizada em lugar do uso de terras (Ono e Cuello, 2007).

A produção de biomassa, a partir do metabolismo fotossintético, em escala comercial é normalmente feita em tanques abertos cuja eficiência é severamente questionada. Embora seja uma configuração de fácil operacionalidade, as produtividades são baixas devido a variação na temperatura e intensidade luminosa devido aos ciclos de luz (dia/noite) e estações do ano (verão, outono, inverno e primavera), tornando-se viável comercialmente apenas para compostos de elevado valor (Sierra et al., 2007; Traviesco et al., 2001; Ogbonna et al., 1996). Por outro lado, sistemas fechados são caracterizados por elevadas eficiências fotossintéticas associadas a maior precisão e controle das variáveis operacionais, menor risco de contaminação e minimização das perdas de água por evaporação (Jacob-Lopes, 2007; Morais et al., 2007; Munhoz e Guiyesse, 2006). As diferenças entre reatores fechados e reatores abertos estão listadas na Tabela 1.2.

Tabela 1-2: Vantagens e Desvantagens do cultivo de microorganismos em reatores abertos e fechados.

Parâmetro	Reator Aberto	Reator Fechado
Risco de contaminação	Elevado	Baixo
Perda de água por evaporação	Elevado	Baixo
Perda do CO_2 alimentado	Elevado	Baixo
Reprodutibilidade	Variável	Possível
Controle do Processo	Complicado	Menos Complicado
Padronização	Difícil	Possível
Dependência com as chuvas	Dependente	Independente
Manutenção	Fácil	Difícil
Concentração da Biomassa	Baixa	Elevada
Custo de Produção	Baixo	Elevado
Eficiência Fotossintética	Baixa	Elevada
Área necessária	Elevada	Baixa

Fonte: Grobbelaar, 2008; Borowitzka, 1999; Molina Grima et al., 1999.

A partir da década de 90 os fotobiorreatores fechados começaram a ser considerados uma alternativa promissora aos tanques abertos, ganhando relevância em projetos desenvolvidos no Japão

(Hirata et al., 1996; Murakami et al.,1997; Kajiwara et al., 1997). Posteriormente, inúmeros grupos de pesquisas em diversos países visaram elucidar a aplicabilidade destes sistemas na biofixação de CO_2 em biomassa (Ono & Cuello, 2006, Jacob-Lopes et al., 2008), sendo que essa investigação se concentra principalmente em duas áreas: os gases de combustão (normalmente com 10-20% CO_2) e do ar em um espaço fechado (geralmente não mais que 1% de CO_2) (Cheng et al. 2005).

Dentre as configurações mais usadas para fotobiorreatores fechados estão os do tipoo coluna de bolhas e airlift. Além dessas principais configurações, outras vêm sendo propostas, tais como geometria retangular, piramidal, cônica, helicoidal e esférica (Sierra et al., 2007; Stewart & Hessami, 2005; Ogbonna et al., 1996). É necessário destacar que o levantamento cinético de fotobiorreatores em escala de bancada é função de cada espécie de cianobactéria, de maneira que as características de crescimento e fisiológicas de cada microrganismo irão determinar o desempenho do sistema (Garcia-Gonzales et al., 2004) e é a etapa inicial para o desenvolvimento desses processos em larga escala (Stewart & Hessami, 2005).

As culturas podem ser cultivadas em regime de batelada, batelada alimentada ou contínuo, sendo o tipo batelada mais utilizados na produção de cianobactérias/microalgas, devido a simplicidade. Basicamente, a operação em batelada consiste num volume limitado de meio inoculado com um número determinado de células exposto à quantidades adequadas de luminosidade, temperatura, salinidade e nutrientes. Não há entrada nem saída de nutrientes e o próprio crescimento celular produz alterações no meio de cultura (Noronha, 1989) assumindo que as condições de crescimento celular são iguais para todas as células. A curva de crescimento desse tipo de cultura apresenta uma forma sigmoidal (Figura 1.1), caracterizada por diferentes taxas de crescimento relacionado às diferentes fases de crescimento populacional, que pode ser tradicionalmente definido em seis etapas (lag, aceleração, exponencial, desaceleração, estacionária e morte) (Strigul et al., 2009; Nakashima et al., 2000).

I. Fase lag ou de adaptação: fase de adaptação do microorganismo onde o crescimento é reduzido ou não ocorre. Síntese das enzimas necessárias ao metabolismo.

II. Fase de transição: fase que termina com toda a população se dividindo num intervalo regular de tempo.

III. Fase exponencial ou de crescimento: Onde a velocidade específica de crescimento é constante e máxima.

IV. Fase desaceleração: Crescimento celular reduzido em função de diversos fatores que podem incluir desde a exaustão dos nutrientes; auto-sombreamento (aumento da densidade celular provoca a redução da quantidade de luz disponível); auto-inibição pelos metabólitos excretados que podem ter um efeito tóxico e, por isso, inibitório do crescimento.

V. Fase estacionária: Onde a concentração atinge um valor máximo e constate.

VI. Fase de declínio: O valor da concentração celular diminui a uma taxa que excede a taxa de crescimento.

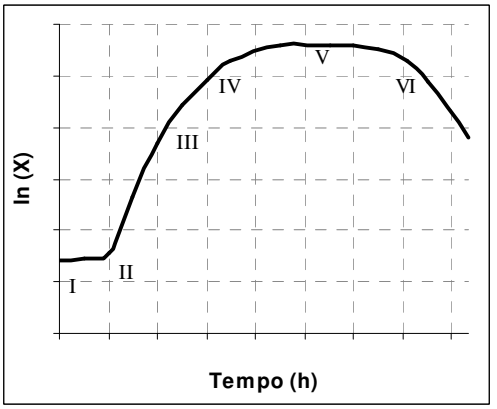

Figura 1.1: Curva característica de crescimento celular em culturas do tipo batelada (Schimidell et al., 2005)

3.4. Efluentes industriais

Segundo o inventario ambiental publicado pela PETROBRAS em 2006 referente a todas as atividades da empresa realizadas no Brasil no ano de 2005, foram geradas 46,3 milhões de toneladas de gás carbônico (CO_2), 222,9 mil toneladas de metano (CH_4) e 980 toneladas de óxido nitroso (N_2O), 152 mil toneladas de óxidos de enxofre (SO_x), 223 mil toneladas óxidos de nitrogênio (NO_x), 119 mil toneladas monóxido de carbono (CO). Em relação aos efluentes líquidos foram produzidos aproximadamente 950 toneladas de óleos e graxas, 1270 toneladas de amônia e 5800 toneladas de carbono orgânico (PETROBRAS, 2006). A figura 1-2 mostra as principais fontes emissoras de gases

do efeito estufa nas unidades da PETROBRAS e a figura 1-3 mostra a elevação das emissões de dióxido de carbono de 1990 até 2001. Além destes, outras substâncias consideradas prejudiciais ao meio ambiente são encontradas nos efluentes do refino de petróleo, em função do tipo de processo utilizado, como compostos fenólicos e metais pesados (Benemann et al., 1997).

METODOLOGIA DE CÁLCULO DAS EMISSÕES ATMOSFÉRICAS | 2002 - 2004

FONTE DE EMISSÃO	POLUENTES	METODOLOGIA DE CÁLCULO | FATOR DE EMISSÃO
EMISSÕES DIRETAS		
QUEIMA DE COMBUSTÍVEIS	CO_2, SO_2	Balanço de Massa
	CH_4, N_2O, MP	Fator de Emissão
	NO_x	Fator de Emissão (*)
TOCHAS (FLARING)	CO_2	Balanço de Massa com Eficiência de Destruição de Hidrocarbonetos Fator de Emissão (**)
	SO_2	Balanço de Massa
	CH_4	Balanço de Massa
	N_2O, MP	Fatores de Emissão
	NO_x	Fator de Emissão
EMISSÕES FUGITIVAS	CH_4	Balanço de Massa e Fatores de Emissão
EMISSÕES EVAPORATIVAS	CH_4	Balanço de Massa e Fatores de Emissão
PROCESSOS	CO_2	Balanço de Massa
	SO_2	Fator de Emissão
	CH_4, N_2O, MP	Fatores de Emissão
	NO_x	Fatores de Emissão (*)
EMISSÕES INDIRETAS (IMPORTAÇÃO DE ENERGIA)		
INDIRETAS	CO_2	Fatores de emissão e porcentagem de energia comprada proveniente de termoelétricas de acordo com o Balanço Nacional
	CH_4	

Figura 1.2: Principais fontes emissoras de gases do efeito estufa da Petrobras.

* Fator de emissão elaborado a partir de características específicas dos processos Petrobras.

** Fator utilizado para estimar as emissões das tochas das unidades de refino localizadas no Brasil

Fonte: Inventário de Emissões de GEE da Petrobras publicado em 2005.

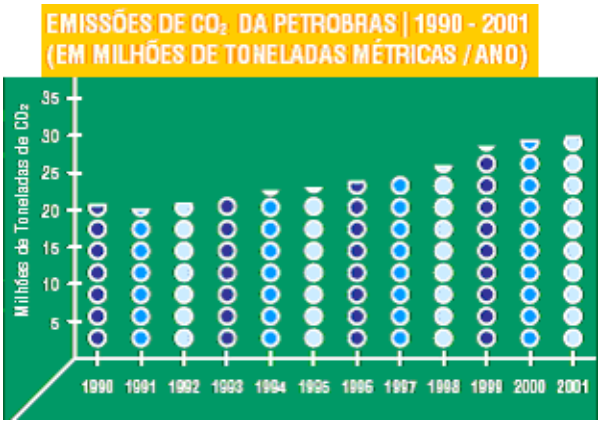

Figura 1.3: Emissões de CO_2 da Petrobras.

Fonte: Inventário de Emissões de GEE da Petrobras publicado em 2005.

Assim, diversos microorganismos veem sendo extensivamente avaliados quanto à possibilidade de aplicação no tratamento biológico de efluentes gasosos associado ao uso dos efluentes líquidos como base de cultivo. Este fato está associado ao complexo metabolismo destes organismos, que podem utilizar distintas rotas metabólicas para a assimilação de compostos poluentes (Lei et al., 2006) e ainda são capazes de assimilar eficientemente variadas formas de compostos nitrogenados, fósforo e metais traços através de processos biológicos e interações fisico-químicas, conforme enunciado na Tabela 1.3 (Munoz e Guieysse, 2006). A Tabela 1.4 apresenta alguns dos trabalhos publicados na área de tratamento de efluentes por microalgas.

O desenvolvimento desta tecnologia resolveria problemas de ordem ambiental, servindo ainda para a produção de insumos (Acien Fernandez et al., 2003). Segundo Maeda e seus colaboradores (1995) a utilização do CO_2 proveniente dos efluentes gasosos para promover o crescimento da biomassa pode ser feita de duas maneiras: (a) usar o CO_2 separado do efluente gasoso ou (b) usar diretamente o efluente gasoso do processo industrial. A utilização direta do efluente gasoso é vantajosa do ponto de vista da economia da energia que seria gasta no processo de separação do CO_2 (Ono e Cuello, 2004), por outro lado o uso direto pode trazer problemas como altas temperaturas, concentração de CO_2 pouco controlada e a possível presença de SOx e NOx (Benemann et al., 1993).

Tabela 1-3: Principais aplicações das microalgas durante o tratamento de efluentes.

Aplicações	Comentários
Redução de BOD	Microalgas liberam 1,5-1,92 $kg_{O2}/kg_{microalgas}$ produzidas durante o crescimento e taxas de oxigenação fotoautotrófica de 0,48-1,85 $kg_{O2}m^{-3}d^{-1}$ têm sido relatados em escala piloto de lagoas ou de laboratório fotobioreactores no tratamento das águas residuais municipais.
Redução de Nutrientes	Microalgas assimilam uma quantidade significante de nutrientes, pois requerem uma grande quantidade de nitrogênio e fósforo para as proteínas (45–60% da massa seca da microalga), ácidos nucleicos e síntese de fosfolipídeos. A remoção de nutrientes pode ser aumentada pela precipitação de fósforo devido ao aumento de pH durante o processo fotossintético.
Redução de Metais Pesados	Microorganismos fotossintéticos podem acumular metais pesados por adsorção física, adsorção química, troca iônica, ligação covalente, precipitação de superfície, reações redox ou cristalização na superfície da célula. A captação desses metais pela célula muitas vezes envolve o transporte de metais pesados para o interior da célula sendo uma importante ferramenta de defesa para evitar o envenenamento e serve para acumular metais traço essenciais. O aumento do pH associado com o crescimento de microalgas pode aumentar a precipitação de metais pesados.
Redução de Patógenos	Microalgas podem reforçar a desativação de patógenos através do aumento do pH, da temperatura e da concentração de oxigênio dissolvido do efluente.
Remoção de poluentes heterotróficos	Certas microalgas verdes e cianobactérias são capazes de utilizar compostos tóxicos recalcitrantes como fontes de carbono, nitrogênio, enxofre ou fósforo.
Produção de Biogas	Produção de CH4 a partir da digestão anaeróbica de biomassa.
Monitoramento de Toxicidade	As microalgas são utilizadas em testes de toxicidade ou em estudos de ecologia microbiana por serem indicadores sensíveis de mudanças ecológicas.

Fonte: adaptado de Muñoz e Guieysse (2006).

Tabela 1-4: Principais aplicações das microalgas durante o tratamento de efluentes.

Microorganismo	Meio de Cultivo	Resultados	Referência
Nostoc muscorum *Anabaena subcylindrica*	esgoto municipal, água residuária da produção de sal e soda e da produção de papel (todos esterilizados).	Revelaram que o efeito do cultivo em esgoto municipal foi favorável para ambos os microorganismos elevando o crescimento celular e a quantidade de pigmentos encontrados nas células. A água resíduária da produção de sal favoreceu esses mesmos pontos apenas para a *N. muscorum* e nos outros casos os valores foram semelhantes entre o efluente e o meio sintético de cultivo.	El-Sheekh et al., 2004
Phormidium valderianum	ASN III enriquecido com soluções aquosas de 25, 50, 75 e 100 mg/l de fenol e acrescido ou não de nitrogênio.	O crescimento foi inibido em todos os testes sem nitrogênio e nos testes com concentração superior a 50 mg/L de fenol. Observou-se uma remoção de 38 mg/L de fenol durante o teste com 50 mg/L.	Shashirekha et al. 1997.
Arthrospira platensis	Efluente da produção de leite.	O crescimento celular foi rápido e sem inibição em até 75 mg/l de NH_3-N. Apontam produtividade de 70 g/m³/dia.	Lincoln et al., 1995.
Scenedesmus bicellularis	Efluente municipal proveniente do tratamento secundário.	Melhor desempenho do microorganismo e maiores quantidades de clorofila em condições de maior disponibilidade de CO_2.	Kaya et al., 1996
Chlorella vulgaris	Tratamento de esgoto primário.	Foi observada uma significativa influencia da concentração inicial do microorganismo na eficiência de remoção de NH^{4+} e PO_4^{3-}.	Lau et al., 1995
Snedesmus incrassatulus	Bold's Basal medium contendo metais pesados (Cromo VI, Cádmio II e Cobre II).	O microorganismo foi capaz de remover entre 25 e 78% dos metais adicionados.	Peña-Castro
Chlorella vulgaris	Efluente líquido e gasoso de uma indústria de aço.	Estudaram o tratamento simultâneo do efluente líquido (fonte de nutrientes) e gasoso (fonte de CO_2). Os resultados demonstraram que uma vazão de efluente líquido de 11233m^3/dia poderia promover a fixação de 23100 kg CO_2/dia e que aproximadamente 12430 kg de biomassa de microalgas poderiam ser produzidos diariamente.	Yun et al., 1997

3.5. Razão C/N e N/P e adaptação de meios de cultivo

A fixação fotossintética de carbono (C) é acompanhada pela fixação de outros elementos químicos como nitrogênio (N) e fósforo (P). A razão de consumo entre esses elementos segue a estequiometria desses elementos na formação da biomassa do microorganismo. Se o consumo de carbono for elevado com relação ao nitrogênio pode estar ocorrendo a formação de material orgânico rico em carbono, fixação de N_2 ou gerar limitação por falta de nitrogênio (Mei et al., 2005), Nesse último caso, o microorganismo não estará apto a produzir as enzimas para utilizar o carbono. Entretanto se existir excesso de nitrogênio, particularmente na forma de amônia, ele poderá inibir o crescimento do microorganismo (Fontenot et al., 2006).

Normalmente, a razão C/N mássica requerida para o desenvolvimento desses microorganismos é 20/1 ou 30/1 (Fontenot et al., 2006). Para a otimização do meio de cultivo que utilize um efluente industrial, além das razões C/N e N/P podem ser testadas diferentes condições de diluição, como realizado por Kang e seus co-autores (2006) no tratamento de efluente primário de esgoto. Eles realizaram testes em diferentes diluições, em água do mar estéril, nas condições de 50%, 25%, 12,5% e 0%, obtendo um maior crescimento para a condição de 25% em função da disponibilidade dos nutrientes existentes nessa condição.

Um processo apropriado de adaptação a um novo meio envolve o esgotamento das reservas intracelulares originais do inoculo e o ajuste de sua maquinaria fisiológica às novas fontes de nutrientes. Nas experiências realizadas em laboratório, para estudar o efeito de um novo meio, o inoculo pode ser crescido em cinco ou seis gerações do meio a ser testado, tendo como controle o meio padrão. Esse tempo é necessário para que as reservas intracelulares não influenciem as taxas de crescimento da cultura e para que ocorra um ajuste completo ao novo ambiente químico. A importância que deve ser dada aos procedimentos de adaptação é evidente, pois alguns meios alternativos podem gerar um bom crescimento em curto prazo, mas são incapazes de manter o crescimento por um número razoável de gerações tornando insustentável a aplicação comercial desses meios. (Voltolina et al.,1998).

3.6. Aplicações da biomassa

Sob a denominação microalgas estão incluídos organismos com dois tipos de estrutura celular: estrutura procariótica, com representantes nas divisões Cyanfhyta (cianobactérias) e Prochlorophyta, estrutura celular eucariótica, com presentantes nas Divisões Chlorophyta, Euglenophyta, Rhodophyta, Haptophyta, Cryptophyta e Dinophyita (Derner, et al., 2006). As microalgas, sob o ponto de vista biotecnológico, não constituem grupo de microorganismos muito estudado. Dentre as dez mil espécies de microalgas que se acredita existirem, pouco mais de mil linhagens são mantidas em coleções ao redor do mundo e apenas algumas centenas foram investigadas por seu conteúdo químico e somente uma pequena quantidade tem sido cultivada em escala industrial. Por serem pouco exploradas, elas representam rica oportunidade para novas descobertas no ramo da biotecnologia (Bertoldi et al., 2008).

Na edição de outubro de 2008 do Current Opinion in Biotechnology, uma série de avaliações foram dedicadas à fornecer dados de como a Biotecnologia podem contribuir para o fornecimento de energia sustentável, diminuir o aquecimento global e poluição, e desempenhar um papel importante na alimentação e problemas de saúde (Angermayr et al., 2009). A Tabela 1.5 mostra os principais produtos e aplicações que podem ser obtidos a partir de microalgas. A Tabela 1.6 mostra o desenvolvimento das tecnologias de produção de microalgas e os principais produtos e preços de varejo.

Tabela 1.5: Principais aplicações das microalgas durante o tratamento de efluentes.

Microorganismo	Grupo	Produto	Aplicação
Spirulina platensis	Cyanobacteria	Ficocianina, biomassa	Alimentos e cosméticos
Chlorella vulgaris	Chlorophyta	Biomassa	Alimentos, suplementos alimentares, substitutos alimentares
Dunaliella salina	Chlorophyta	Carotenoides, β-caroteno	Alimentos, suplementos alimentares
Haematococcus pluvialis	Chlorophyta	Carotenoides, astaxantina	Alimentos, fármacos e substitutos alimentares
Odontella aurita	Bacillariophyta	Ácidos graxos	Fármacos, cosméticos, alimentos para bebes
Porphyridium cruentum	Rhodophyta	Polissacarídeos	Fármacos e cosméticos
Isochrysis galbana	Chlorophyta	Ácidos graxos	Ração para animais
Phaedactylum tricornutum	Bacillariohyta	Lipideos, ácidos graxos	Produção de combustíveis

Fonte: adaptado de Pulz e Grooz, 2004.

Tabela 1.6: Principais produtos, aplicações, valor de varejo e desenvolvimento dos processos produtivos de microalgas.

Produto		Valor de Varejo (U.S. $×106)	Desenvolvimento
Biomassa	Alimentos	1250-2500	Crescendo
	Alimentos funcionais	800	Crescendo
	Aditivos Alimentares	300	Crescimento Acelerado
	Aqüicultura	700	Crescimento Acelerado
	Condicionador de solo		Promissor
Pigmentos	Astaxantina	<150	Começando
	Ficocianina	>10	Estagnado
	Ficoeritrina	>2	Estagnado
Antioxidantes	B-Caroteno	>280	Promissor
	Tocoferol		Estagnado
Produtos Especiais	Toxinas	1-3	
	Isótopos	>5	

A literatura indica que a principal vantagem no uso de cianobactérias/microalgas no tratamento de efluentes está na biomassa produzida que pode ser utilizada em diversas finalidades, como em ração animal, compostagem, produção de adubo e também na obtenção de uma forma mais nobre de aproveitamento de suplemento alimentar para humanos, seria um ganho adicional ao controle de poluição (Queiroz et al., 1997), ajudando assim a diminuir os custos totais da planta de tratamento (Voltolina, 1998). Nas últimas décadas, programas de seleção de microorganimos tem revelado que cianobacterias formam uma potencial fonte de novos princípios ativos para medicina e fármacos tendo inúmeros compostos já isolados (Mundt et al., 2001).

Bioenergia também é uma possibilidade de uso dessa biomassa (Kang et al., 2006). Para um país tropical como o Brasil, o substituto natural para o petróleo pode ser a biomassa. Além de ser renovável ela reduz a poluição, pois é formada a partir de CO_2 e H_2O, aproveitando a energia solar. Consideremos que 1 ton de biomassa corresponde a aproximadamente 2,9 barris de petróleo (valor calorífico médio do petróleo = 10000 kcal/kg; biomassa base seca = 4000 kcal/kg) e que o Brasil precisa atualmente de 1.800.000 barris de petróleo por dia (90 x 106 ton de petróleo por ano). Isso poderia ser suprido por 225 x 10^6 ton de biomassa por ano. (Schuchardt et al., 2001).

A conversão da energia luminosa na energia elétrica usando microalgas/cianobactérias e seus componentes fotossintéticos foi estudada já que o processo fotossintético é uma reação sofisticada com elevada eficiência. Na primeira etapa da fotossíntese, a passagem da carga na corrente de transporte do elétron ocorre produzindo equivalentes reduzidos. Em uma pilha fotossintética de bio-combustível, usando cianobactéria, tais reduzidos se comunicam com os eletrodos produzindo uma corrente elétrica por ação catalítica da cianobactéria e de mediadores redutivos. Uma corrente elétrica substancial é produzida também no escuro. Neste caso, reduzindo o poder das reservas internas (principalmente glicogênio) que são convertidas similarmente na energia elétrica. O desempenho das pilhas do bio-combustível depende da quantidade de glicogênio acumulada dentro da cianobactéria para fotossíntese. O glicogênio é uma fonte do elétron nas pilhas do bio-combustível que funcionam no escuro. (Yagishita et al, 1996).

Barbosa (2003) cita a possibilidade de aplicação dessa biomassa em alimentos naturais, alimentos funcionais, aditivos alimentares, aqüicultura e condicionador de solo. Ele ainda indica a utilização dos compostos que podem ser obtidos a partir da biomassa: i) corantes e antioxidantes (aditivos alimentares e cosméticos); ii) ácidos graxos (aditivos alimentares); iii) enzimas (alimentos naturais, pesquisa e medicina) e iv) polímeros (aditivos alimentares, cosméticos e medicina).

3.7. Modelagem Preditiva

Apesar das inúmeras aplicações possíveis, apenas algumas poucas espécies de algas e cianobactérias são cultivadas comercialmente devido ao reduzido desenvolvimento dos fotobiorreatores (Degen et al. 2000). O sucesso em escala comercial desta tecnologia é fundamentado na viabilidade técnica e econômica da produção de compostos celulares de alto valor agregado, existindo a necessidade de controlar as variáveis operacionais, diminuir o risco de contaminação e minimização das perdas de água por evaporação (Jacob-Lopes, 2007; Morais et al., 2007; Munhoz e Guiyesse, 2006). Neste sentido, a construção de modelos matemáticos e cinéticos é atividade essencial para o desenvolvimento desses processos, entretanto, existem poucos profissionais especialistas na análise e modelagem da cinética de processos, sendo que a maior parte dos que atuam na área encontra dificuldade na manipulação das informações matemáticas, razão pela qual o processo de geração de modelos é lento e sujeito a erros (Martins e Barral, 2004).

Um modelo de predição é formado por dois componentes, a parte determinística que representa a relação entre a variável de resposta e a(s) variável(is) explanatória(s) e a parte estocástica que representa o quanto a resposta esperada se desvia da resposta observada ou real. A escolha de uma função para descrever uma determinada resposta é um exercício estatístico, haja vista que se está lidando com estimativas da resposta real. Em contraste, o ajuste da função escolhida aos dados é mais um exercício de álgebra, e consiste na determinação dos valores dos parâmetros que melhor ajustam o modelo escolhido ao conjunto de dados coletados (Nakashima et al., 2000). Os modelos matemáticos podem ser classificados em lineares ou não-lineares nos parâmetros. Uma importante conseqüência da não-linearidade de um modelo de regressão é que os estimadores de mínimos quadrados dos seus parâmetros não possuem as propriedades estatísticas desejáveis dos correlatos nos modelos de regressão linear (Neter et al., 1996).

Pode-se considerar que os modelos matemáticos estabelecidos para a microbiologia preditiva possuem três níveis: nível primário ou primeiro nível, nível secundário ou segundo nível e nível terciário ou terceiro nível. O nível primário corresponde a modelos matemáticos que descrevem a mudança do número de microorganismos em função do tempo. O nível secundário envolve equações que descrevem como as respostas dos modelos primários (duração da fase lag, velocidade de crescimento, densidade máxima de população, consumo de substrato) mudam com alterações nos

fatores ambientais. O nível terciário é constituído por programas (softwares) utilizados para resolver os modelos de nível primário e secundário (Juneja et al., 2009; McDonald & Sun, 1999; Whiting e Buchanan, 1993). A Tabela 1.7 apresenta os principais artigos pesquisados sobre modelagem de crescimento celular.

Tabela 1-7: Principais artigos sobre modelagem de predição de crescimento celular.

Descrição	Referencia
Revisão de aspectos básicos de modelagem preditiva: Modelos de nível primário, secundário, terciário, validação da metodologia, comparação entre métodos e aplicações.	Nakashima et al., 2000.
Revisão de aspectos básicos de modelagem preditiva: Modelos de nível primário, secundário, terciário, validação da metodologia, comparação entre métodos e aplicações.	McDonald e Sun, 1999.
Desenvolvimento de novos modelos de predição de crescimento celular comparados com o modelo Baranyi.	Van Impe et al., 2004
Comparação entre o desempenho dos modelos Gompertz Modificado, Logístico e Baranyi. Observou-se que os Modelos Gompertz Modificado e Logístico apresentaram valores semelhantes e significativamente diferentes aos encontrados utilizando o modelo Baranyi. Comparando esses valores com os obtidos através da curva ln (X) x t foi observada maior correlação com os modelos Gompertz Modificado e Logístico.	Perni et al., 2005.
Avaliação de Modelos de predição e construção de superfícies de resposta para a predição dos parâmetros de crescimento celular da *Clostridium sporogenes.* Apresentação de modelos de validação estatística e matemática dos modelos de crescimento testados.	Dong et al., 2007
Apresentação de modelos de estimativa de produtividade celular em processos contínuos em função de diferentes períodos iniciais de batelada.	Muller-Feuga et al., 2003.
Validação dos modelos Gompertz Modificado e Logístico para predição de parâmetros de crescimento celular da *Pediococcus acidilactici.*	Chowdhury, et al., 2006.

3.7.1 Crescimento de biomassa – nível primário

Os modelos de crescimento celular podem ser obtidos por técnicas de regressão não linear. O modelo logístico descreve o crescimento celular de populações de microorganismos em função da concentração inicial e final, tempo e taxa de crescimento. A função original foi desenvolvida por Pearl e Reed (1920), sendo baseada nos ensaios de Verhulst (1838). A modelo Logístico está expresso:

$$y = \frac{A + C}{1 + exp^{(-B(t-M))}} \quad (1)$$

A equação original de Gompertz não considera a fase lag, como é o caso de sua forma modificada, mas apenas o aumento da densidade de células, uma vez iniciado o crescimento exponencial (McMeekin et al., 1993). Um grande número de funções tem sido derivadas do modelo clássico de Gompertz que está apresentado na equação abaixo:

$$y = A + Cexp^{(-exp[B(t-M)])} \quad (2)$$

Zwietering e seus co-autores re-parametrizaram a equação de Gompertz para incluir três parâmetros biológicos relevantes: tempo da fase lag (λ), taxa de máximo crescimento específico ($\mu_{máx}$) e máxima concentração celular ($X_{máx}$) (Zwietering et al., 1990). Trata-se de uma função exponencial dupla, que descreve uma curva sigmóide assimétrica (Whitinig e Buchanan, 1997). O modelo está representado abaixo:

$$y = C.exp^{-exp\left[\frac{\mu_{max}.exp(1)}{C}(\lambda - t)+1\right]} \quad (3)$$

O modelo de Gompertz modificado e o logístico são os que mais habilitados para descrever uma curva de crescimento contendo as três fases mais importantes (lag, exponencial e estacionaria). No caso de curvas incompletas (sem a fase estacionária) os modelos podem não ser capazes de descrever a curva (Juneja et al., 2009). Apesar da vasta utilização do modelo de Gompertz modificado, a equação possui falhas de acordo com Whitining e Buchanan (1997). A fase lag não é paralela ao eixo das abcissas e a assíntota matemática está fora da escala em tempo negativo. A equação não é uma reta e, portanto, não apresenta um período de aumento linear durante a fase de crescimento exponencial, como é observado com a maioria das curvas de crescimento.

Para solucionar esses problemas e fornecer uma base mais mecanística ou biológica, o modelo proposto por Baranyi e Roberts (1994) incluíram uma fase de crescimento exponencial linear, μ(x) e uma fase lag determinada por uma função de ajuste A(t) (Baranyi et al., 1993; Baranyi and Roberts, 1994; Baranyi, 1997):

$$y = \mu_{max} A(t) - ln\left(1 + \frac{exp^{\mu_{max} \cdot A(t)} - 1}{exp^{c}}\right) \quad (4)$$

onde

$$A(t) = t + \frac{1}{\mu_{max}} ln\left(exp^{-\mu_{max} \cdot t} + exp^{-\mu_{max} \cdot \lambda} - exp^{-\mu_{max} \cdot (t+\lambda)}\right) \quad (5)$$

O modelo de Morgan é outra expressão que pode ser utilizada para descrever o crescimento celular (Morgan et al., 1975):

$$y = \frac{A.t^{v}}{K^{v} + t^{v}} \quad (6)$$

3.7.2 Consumo de substrato

A relação entre o crescimento celular e o consumo de substrato é muito complexa para ser descrita em termos das leis físicas fundamentais, por isso faz-se simplificações fundamentais para representar os modelos de consumo de substrato. As aproximações mais usadas são a de um único substrato limitante e o os outros em excesso (assim apenas a concentração de um substrato é levada em consideração nos cálculos), presença de um inibidor, células representadas como um corpo homogêneo (as diferenças dos múltiplos componentes e comportamentos celulares são desconsideradas quando não interferem no processo cinético), apenas um componente é importante na cinética do processo (desconsidera-se a heterogeneidade das células) e, portanto, considera-se a população como um ser homogêneo e com uma única propriedade. Essa perspectiva, apresentada por Bailey e Ollis, 1986, classifica as abordagens para os sistemas microbianos de acordo com o número de componentes usados na representação celular, e se as células são ou não vistas como uma coleção heterogênea de entidades discretas, como realmente o são ou, ao contrário, como algum tipo de média celular, a qual conduz ao mesmo conceito de um componente em solução. Representações celulares multicomponentes são chamadas estruturadas, e representações simples são designadas não-estruturadas. Considerar as células de uma forma discreta e heterogênea é um enfoque segregado, enquanto a perspectiva não-segregada considera propriedades celulares médias (Blanch e Clark, 1997; Shuller e Kargi, 1992; Bailey e Ollis, 1986).

Um dos mais simples modelos de crescimento que incluem o efeito da concentração de nutrientes é o modelo desenvolvido por Jacques Monod baseado em observações do crescimento da *E. coli* em diferentes concentrações de glicose. Neste modelo assume-se que apenas um substrato limita o crescimento, de forma que somente a mudança na concentração desse substrato é relevante (normalmente uma fonte de C). A forma da equação de Monod é similar à de Michaelis_Menten para cinética enzimática (Monod, 1949) e está expressa na equação 7.

$$\mu_g = \mu_{max} \frac{S}{k_s + S} \qquad (7)$$

Uma modificação simples pode ser feita na equação de Monod quando se trata de culturas de elevada densidade celular.

É observado experimentalmente que a taxa de crescimento diminui com altos valores da concentração inicial substrato (S_0), devido à influência da força iônica, pressão osmótica, ou pela sobrecarga dos sistemas de transporte da membrana. Além disso, os metabólicos que podem não ser tóxicos em níveis normais, podem acumular. Estes efeitos podem ser vistos na equação modificada de Monod, onde o termo k_{so} é adicionado para reduzir a taxa de crescimento (BLANCH & CLARK, 1997). Esta modificação é normalmente utilizada quando o consumo de substrato ou a liberação de resíduos tóxicos são rápidos, a concentração celular é elevada ou quando a acumulação torna-se significativa.

$$\mu_g = \mu_{max} \frac{S}{k_s + k_{s0}.S_0 + S} \qquad (8)$$

Outras equações têm sido propostas e, dependendo da forma da curva μ-S, uma dessas equações pode ser mais plausível que as outras. A equação Teissier, Equação 9, possui duas constantes (μ_{max}, k_s). A equação Moser, Equação 10, apresenta três constantes (μ_{max}, n, k_s) e quando n=1 ela é equivalente à equação de Monod. Já a equação de Contois, Equação 11, possui uma constante de saturação proporcional a concentração celular, descrevendo a limitação do substrato em altas densidades celulares. Em acordo com essa equação, a taxa específica de crescimento celular diminui com a queda da concentração de substrato no meio e eventualmente pode se tornar inversamente proporcional a concentração celular presente no meio.

$$\mu_g = \mu_{max} \left(1 - e^{S.k}\right) \qquad (9)$$

$$\mu_g = \mu_{max} \frac{S^n}{k_s + S^n} \tag{10}$$

$$\mu_g = \mu_{max} \frac{S}{k_s.X + S} \tag{11}$$

Vários modelos estão disponíveis na literatura para representar o crescimento celular influenciado por um substrato que também atua como inibidor. Webb apresentou seu modelo, Equação 12, em 1964, introduzindo em seu modelo uma constante de inibição auxiliar além das constantes de inibição e de saturação. Aiba e seus co-autores, 1968, apresentaram o modelo mais simples em termos de quantidade de parâmetros, mas ele apresenta uma exponencial, Equação 13. O modelo Andrews, Equação 14, foi apresentado em (1968), sendo bem mais simples em termos matemáticos quando comparado aos outros (Andrews, 1968). Em 1969, Yano e Koga apresentarem um modelo, Equação 15, que conta com uma constante de inibição auxiliar quadrada. Neste modelo a concentração do substrato é elevada ao cubo fazendo com que o erro em uma medida influencie ainda mais no resultado. As equações 16 e 17 representam os modelos Ierusalimsky e Chen, respectivamente.

$$\mu = \mu_{max} \frac{S\left(1 + \frac{S}{k_i}\right)}{S + k_s + \frac{S^2}{k_i}} \tag{12}$$

$$\mu = \mu_{max} \frac{S}{k_s + S} e^{-S/k_i} \tag{13}$$

$$\mu = \mu_{max} \frac{1}{1 + \frac{k_s}{S} + \frac{S}{k_i}} \tag{14}$$

$$\mu = \mu_{max} \frac{S}{k_s + S\left(1 + \frac{S}{k_i} + \frac{S^2}{k_{i2}^2}\right)} \tag{15}$$

$$\mu = \mu_{max} \frac{S}{k_s + S} exp\left(-\frac{S}{k_i}\right) \quad (16)$$

$$\mu = \mu_{max} \frac{S\left(1 + \frac{S}{k_i}\right)}{k_s + S + (k_{s2}.S)^2} \quad (17)$$

Ohtaguchi e Wijanarko (2004) apresentam a modificação dos modelos de inibição (Equações 18 a 23) para que seja possível usa-los quando a alimentação do substrato, dióxido de carbono, é contínua (Ohtaguchi e Wijanarko, 2004).

$$q_{CO2} = q_{CO2\_max} \frac{X\left(k_X + \frac{X}{k_{I,2}}\right)}{k_X + X\left(k_X + \frac{X}{k_I}\right)} \quad (18)$$

$$q_{CO2} = q_{CO2\_max} \frac{Xe^{-X/k_I}}{X + k_X} \quad (19)$$

$$q_{CO2} = q_{CO2\_max} \frac{X}{X\left(k_X + \frac{X}{k_I}\right) + k_X} \quad (20)$$

$$q_{CO2} = q_{CO2\_max} \frac{X}{k_X + X\left(k_X + \frac{X}{k_I} + \frac{X^2}{k_{I,2}^2}\right)} \quad (21)$$

$$q_{CO2} = q_{CO2\_max} \frac{X}{k_X + X} \frac{1}{k + \frac{X}{k_I}} \quad (22)$$

$$q_{CO2} = q_{CO2\_max} \frac{X\left(k + \frac{X}{k_I}\right)}{X + k_X + \left(k_{X2} X\right)^2} \quad (23)$$

3.7.3 Resolução e validação dos modelos

A resolução dos modelos pode ser feita via método numérico (soma dos erros ou estimativa dos quadrados) (Rodriquez et al., 2005). Existem vários procedimentos numéricos interativos para a obtenção das estimativas de mínimos quadrados dos parâmetros de um modelo não-linear. Entre eles pode ser citado o Método de Gauss-Newton (Neter et al., 1996).

O ajuste dos dados experimentais aos modelos é um processo interativo, sendo a resolução atrelada à escolha do ponto inicial de cada parâmetro. Uma escolha errada desse valor pode comprometer a solução do modelo levando à respostas sem significado físico ou mesmo à soluções divergentes. A escolha desse ponto inicial pode ser problemática quando não se conhecem os dados e os parâmetros de maior influência do modelo, para isso pode-se alterar valores desses parâmetros e verificar as mudanças nos resultados e na inclinação da curvatura do modelo (Motulsky & Ransnas, 1987; Motulsky & Christopoulos, 2003). O ponto inicial deve ser testado em uma ampla faixa de valores para garantir que não seja encontrado um ponto de mínimo local, situação que é comum quando se testa muitos pontos ou os dados foram coletados numa faixa inapropriada para aquele modelo (Neeleman, 2002).

A validação estatística dos modelos pode ser feita através das expressões listadas abaixo, onde o erro médio quadrático, RMSE, representa a adequação dos modelos aos dados experimentais. Quanto melhor o ajuste do modelo, menor será o valor do RMSE calculado. O erro padrão de predição (%SEP) mostra que o modelo será melhor quanto mais esse parâmetro se aproximar do zero. O fator bias (Bf) e o fator de exatidão (Af) fornecem uma indicação objetiva do desempenho do modelo. Se o fator bias é menor que 1 significa que os valores preditos são menores que os observados, e se é maior que 1 significa que os valores preditos são maiores que os observados. De acordo com Ross (1996), modelos que descrevem a taxa de crescimento com Bf em uma faixa de 0,9–1,05 são considerados bons, de 0,7–0,9 ou 1,06–1,15 são considerados aceitáveis e <0,7 ou >1,15 não podem ser utilizados. Os valores do fator de exatidão representam quanto os preditos diferem dos observados em média, conforme aumenta esse valor, o modelo é menos exato na média, isto é, menor a exatidão da estimativa (Baranyi et al., 1993; Hervás et al., 2001; Ross, 1996; Garcia-Gimeno et al., 2005; Zuzera-Cosano et al., 2006).

$$RMSE = \sqrt{\frac{\sum (obs - pred)^2}{n}} \qquad (24)$$

$$\%SEP = \frac{100}{média\ obs}\sqrt{\frac{\sum(obs - pred)^2}{n}} \quad (25)$$

$$B_f = 10^{\frac{\sum log(pred/obs)}{n}} \quad (26)$$

$$A_f = 10^{\frac{\sum |log(pred/obs)|}{n}} \quad (27)$$

A escolha entre diferentes modelos deve seguir alguns critérios básicos: (i) ajuste do modelo aos dados; (ii) número e significado físico dos parâmetros envolvidos em cada modelo (melhor a minimização da quantidade de parâmetros); (iii) faixa em que o modelo pode ser aplicado; (iv) estudo do comportamento do erro obtido (a distribuição dos pontos da curva devem seguir uma gaussiana, a distancia média entre os pontos deve ser a mesma e deve existir uma aleatoriedade entre os pontos acima e abaixo da curva) (v) facilidade de uso do modelo e (iv) significado físico dos resultados (Motulsky & Christopoulos, 2003; Nakashima, et al., 2000; Motulsky & Ransnas, 1987).

4 Referencias

ACIÉN FERNÁNDEZ, F. G., ALÍAS, C. B., GARCIA-MALEA, M. C., FERNÁNDEZ SEVILLA, J. M., IBÁNEZ GONZÁLEZ, M. J., NÚNEZ GÓMEZ, R., MOLINA GRIMA, E. Assessment of the production of 13C labeled compounds from phototrophic microalgae at laboratory scale, Biomolecular Engineering, 20, p.149-162, 2003.

AIBA, S., HUMPHREY, A.E, MILLIS, N.F. Biochemical engineering. Academic press, 1st edition, 1968.

AIBA, S., SHODA, M., NAGALANI, M. Kinetics of product inhibition in alcohol fermentation. Biotechnol Bioeng., 10, 845-964, 1968.

ANAND, N. Cyanobacterial taxanomic, classical concepts and modern trends. Cyanobacterial biotechnology , p. 337-340, 1998.

ANDREWS, J.F. A mathematical model for the continuous culture of microorganisms utilizing inhibitory substance. Biotechnology. Bioengineering, 10, p.707-723, 1968.

ANGERMAYR, S.A., HELLINGWERT, K.J., LINDBLAD, P., MATTOS, M.J.T.M. Energy biotechnology with cianobactéria. Current opinion in Biotechnology, 20, 257-263, 2009.

ARAÚJO, O.Q.F., GOBBI, C.N., CHALOUB, R.M., COELHO, M.A.Z. Assessment of the Impact of Salinity and Irradiance on the Combined Carbon Dioxide Sequestration and Carotenoids Production by Dunaliella salina: A Mathematical Model. Biotechnology and Bioengineering, 102, 2, 425-435, 2008.

AUSTRALIAN GOVERNMENT – BUREAU OF METEOROLOGY. Acessado em 17/08/2009. Disponível em http://www.bom.gov.au/info/GreenhouseEffectAndClimateChange.pdf.

AVILA, A.M.H. Uma síntese do quarto relatório do IPCC. Revista Multiciência, Campinas, 2007.

BAILEY, J.E., OLLIS, D.F. Biochemical engineering fundamentals. New York, N.Y, McGraw-Hill Inc., 1977.

BARANYI, J. Simple is good as long as it is enough. Food Microbiology, 14, p.189–192, 1997.

BARANYI, J., ROBERTS, T.A. A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology, 23, p.277–294, 1994.

BARANYI, J., ROBERTS, T.A., MCCLURE, P. A non-autonomous differential equation to model bacterial growth. Food Microbiology, 10, p.43–59, 1993.

BARBOSA, M.J.G.V. Microalgal photobioreactors: scale up and optimization. Holanda, Wageningen University, 2003. Tese de doutorado em Ciências.

BENEMAN, J., HUGHES, E. Biological fossil CO_2 mitigation. Energy Conversion and Management, 36, p.5467-5473, 1997.

BENEMANN, J.R. Utilization of carbon dioxide from fossil fuel—burning power plants with biological system. Energy Conversion and Management, 34(9/11), p.999–1004, 1993.

BERTOLDI, F.C., SANT'ANNA, E., OLIVEIRA, J.L.B. Revisão: Biotecnologia de Microalgas. B.CEPPA, Curitiba, 26, 1, 2008.

BILANOVIC, D., ANDARGATCHEW, A., KROEGER, T., SHELEF. G.. Freshwater and marine microalgae sequestering of CO_2 at different C and N concentrations – Respnse surface methodology analysis. Energy Conversion and Management, 50, p.262–267, 2009.

BLANCH, H.W., CLARK, D.S. Biochemical engineering. Marcel Dekker, Inc., New York, 1997.

BOROWITZKA, M. IN: BOROWITZKA, M. & BOROWITZKA, L. Micro-algal Biotechnology, 2º Ed., Sydney, p.477, 1994.

CHENG, L., ZHANG, L., CHEN, H., GAO, C. Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor. Separation and Purification Technology, 50, p.324–329, 2006.

CHOWDHURY, B.R., CHAKRABORTY, R., CHAUDHURI, U.R. Validity of modified Gompertz and Logistic models in predicting cell growth of Pediococcus acidilactici H during the production of bacteriocin pediocin AcH. Journal of Food Engineering, 80, 1171-1175, 2007.

COLLINS, A.M., XIN, Y., BLANKENSHIP, R.E.. Pigment organization in the photosynthetic apparatus of *Roseiflexus castenholzii.* Biochemica et Biophisica Acta, Article in press.

CONTOIS D E. Kinetics of bacterial growth: relationship between population density and specific growth rate of continuous culture. J Gen Microbiol.,**21,** 40–50, 1959.

DE LORENZO, M. Relação de nutrientes de fatores ambientais na ocorrência de cianobactérias *Aphanothece sp.,* ao redor da cidade do Rio Grande. FURG: Curso de Oceanografia. (1995). (Trabalho de Graduação).

DEGEN, J., UEBELE, A; RETZE, A., SCHMID-STAIGER, U., TRÖSCH, W. A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. Journal. of Biotechnoogyl., 92, 2, 28, p.89-94, 2001.

DERNER, R.B., OHSE, S., VILLELA, M., CARVALHO, S.M., FETT, R.. Microalgas, produtos e aplicações. Ciência Rural, Santa Maria, 36, n.6, p.1959-1967, 2006.

DONG, Q., TU, K., GUO, L., LI, H., ZHAO, Y., 2007. Response surface model for prediction of growth parameters from spores of Clostridium sporogenes under different experimental conditions. Food Microbiology, 24, 624-632.

EDWARDS, V.H. The influence of high substrate concentrations on microbial kinetics. Biotechnol. Bioeng., 12, n.5, p.679-712, 1970.

EL-SHEEKH, M., EL-SHOUNY, W.A., OSMAN, M.E.H., EL-GAMMAL, W.E. Growth and heavy metals removal efficiency of Nostoc muscorum and Anabaena subcylindrica in sewage and industrial wastewater effluents. Environmental Toxicology and Pharmacology, 19, 357-365, 2005.

ESTEVES, F.A. Fundamentos de limnologia. Rio de Janeiro: ed. Internacional, p.570,1988.

FALKOWSKI, P.G. Photosynthesis: The paradox of carbon dioxide efflux. Current Biology., 7, p.637-639, 1997.

FONTENOT, Q., BONBILLAIN, C., KILGEN, M., BOOPATHY, R. Effects of temperature, salinity, and carbon:nitrogen ratio on sequencing batch reactor treating shrimp aquaculture wastewater. Bioresource Technology, 2006.

GARCIA-GONZALEZ, M., MORENO, J., MANZANO, C., FLORENCIO, J., GUERRERO, M. Production of Dunaliella salina biomass rich in 9-cis-β-carotene and lutein in closed tubular photobioreactor. Journal of Biotechnology. *In pre*ss, 2004.

GARCÝA-GIMENO, R.M., BARCO, E., RINCON, F., ZURERA-COSANO, G. Response surface model for estimation for *Escherichia coli* growth under different experimental conditions. Journal of Food Science; 70, p.30–36, 2005.

GROBBELAAR, J.U. Factors governing algal growth in photobioreactors: the "open" versus "closed" debate. Journal of Applied Phycol., 2008.

GROBBELAAR, J.U. Physiological and technological considerations for optimizing mass algal cultures. Journal Applied Phycoloy, 12, p.201-206, 2000.

HALPERIN, R; ZULPA, D.C.; ZACCAR, M. C. Contenido proteico de *Aphanothece stagnina (Sprengel).* A Braun (Cyanophyta, chorococcaceae) Phisis Seccion B, Buenos Aires, 33, nº 87, 159-164, 1974.

HERVÁ S, C., ZURERA, G., GARCÍA, R.M., MARTÝNEZ, J. Optimization of computational neural network for its application to the prediction of microbial growth in foods. Food Science Technoloy, 7, p.159–163, 2001.

HIRATA, S.; HAYASHITANI, M.; TONES, S. Carbon Dioxide Fixation in Batch Culture of Chlorela sp. Using a Photobioreactor with a Sunlight-Collection Device. Journal of Fermentation and Bioengineering. 81, n.5, p.470-472, 1996.

HSUEH, H.T., LI, W.J., CHEN, H.H., CHU, H. Carbon bio-fixation by photosynthesis of *Thermosynechococcus sp.* CL-1 and *Nannochloropsis oculta*. Journal of Photochemistry and Photobiology B: Biology, 95, p.33–39, 2009.

INPE, Acessado em 17/08/2009. Disponível em: http://videoseducacionais.cptec.inpe.br/swf/mud_clima/02_o_efeito_estufa/02_o_efeito_estufa.swf

IPCC, Intergovernmental Panel on Climate Change. Carbon dioxide capture and storage. http://www.ipcc.ch/, 2007.

JACOB-LOPES, E. Seqüestro de dióxido de carbono em fotobiorreatores. Campinas: Faculdade de Engenharia Química, Universidade Estadual de Campinas, 2007 (Tese de doutorado).

JACOB-LOPES, E., LACERDA, L. M. C. F., FRANCO, T. T. Biomass production and carbon dioxide fixation by *Aphanothece microscopica Nägeli* in a bubble column photobioreactor, Biochemical Engineering Journal, 40, p.27-34, 2007.

JACOB-LOPES, E., SCOPARO, C.H.G., FRANCO, T.T. Rates of CO2 removal by *Aphanothece microscopica Nägeli* in tubular photobioreactors. Chemical Engineering and Processing, 47, p.1365–1373, 2008.

JACOB-LOPES, E., SCOPARO, C.H.G., LACERDA, L. M. C. F., FRANCO, T. T. Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactores. Chemical Engineering and Processing: Process Intensification. V. 48, p306-310, 2009.

JEAN-BAPTISTE, P., DUCROUX, R. Energy policy and climate change. Energy Policy 31, 155–166, 2003.

JEON, Y.C., CHO, C.W., YUN, Y.S. Measurement of microalgal photosynthetic activity depending on light intensity and quality, Biochemical Engineering Journal, 27, p.127-131, 2005.

JUNEJA, V.K., MELENDRES, M.V., HUANG, L., SUBBIAH, J., THIPPAREDDI, H. Mathematical modeling of growth of *Salmonella* in raw ground beef under isothermal conditions from 10 to 45ºC. International Journal of Food Microbiology, 131, p.106-11, 2009.

KAJIWARA, S., YAMADA, H., NARUMASA, O. Designof the bioreactor for carbon dioxide fixation by Synechococcus PCC7942. Energy, Conversion, Management, 38, p.529-532, 1997.

KAYA, V.M., GOULET, J., NOÜE, J., PICARD, G. Effect of intermittent CO2 enrichment during nutrient starvation on tertiary treatment of wastewater by alginate-immobilized Scenedesmus bicellularis. Enzyme and Microbial Technology, 18, 550-554, 1996.

KANG, C.D., AN, J.Y., PARK, T.H., SIM, S.J. Astaxanthin biosynthesis from simultaneous N and P uptake by the green alga *Haematococcus pluvialis* in primary-treated wastewater; Biochemical Engineering Journal, 31, p.234–238, 2006.

KILBANE,. J.J. Microbial biocatalyst developments to upgrade fossil fuels. Current Opinion in Biotechonology, 17, p.305-314, 2006.

LAU, P.S., TAM, N.F.Y., WONG, Y.S. Effect of algal density on nutrient removal from primary settled wastewater. Environmental Pollution, 89, 59-66, 1995.

LE TREUT, H., R. SOMERVILLE, U. CUBASCH, Y. DING, C. MAURITZEN, A. MOKSSIT, T. PETERSON AND M. PRATHER, 2007: Historical Overview of Climate Change. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [SOLOMON, S., D. QIN, M. MANNING, Z. CHEN, M. MARQUIS, K.B. AVERYT, M. TIGNOR AND H.L. MILLER (EDS.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

LEI, A.P., HU, Z.L., WONG, Y.S., TAM, N.F.Y. Removal of fluoranthene and pyrene by different microalgal species. Bioresource Technology, n.98.p.273-280, 2007.

MAEDA, K., OWADA, M., KUMURA., OMATA, K., KARUBE,. I. CO_2 fixation from the flue gas on coal-fired thermal power plant by microalgae, Energy convers. Mgmt, 36, n6-9, p 717-720, 1995.

MARTÍNEZ, M.E., SÁNCHEZ, S., JÍMENEZ, J.M., YOUSFIS, F.E., MUÑOZ, L. Nitrogen and phosphorus removal from urban wastewater by the microalga Scenedesmus obliquus. Bioresource Technology, n.73,p.263-272, 2000.

MARTINS, F.P.R., BARRAL, M.F., Ferramenta de apoio à formulação simbólica de modelos cinéticos microbianos não estruturados. Revista brasileira de ensino de bioquímica e biologia molecular, 2004.

MCDONALD, K., SUN, D.W. Predictive food microbiology for the meat industry. International Journal of Food Microbiology, 52, p.1-27, 1999.

MCMEEKIN, T.A., OLLEY, J.N., ROSS, T., RATKOWSKY, D.A. Predictive Microbiology: Theory and Application, Wiley, New York, 1993.

METTING, F.B. Biodiversity and application of microalgae. Journal of Industrial Microbiology, 17, 477-489, 1996.

MEI, Z.P., LEGENDRE, L., TREMBLAY, J.E., MILLER, L.A., GRATTON, C.L., YAGER, P.L., GOSSELIN, M. Carbon to nitrogen (C:N) stoichiometry of the spring-summer phytoplankton bloom in the North Water Polynya (NOW). Deep-Sea Research, 1, n.52, p.2301-2314, 2005.

MOLINA GRIMA, E.; FERNANDÉZ, F.G.A.; CAMACHO, F.G.; CHISTI, Y. Photobioreactors: light regime, mass transfer, and scale up. Journal of Biotechnology, n.70, p.231-247, 1999.

MONOD, J. The growth of bacterial cultures. Ann. Rev. Microbiol., 3, p.371-394, 1949.

MONTOYA & GOLUBIC, S. Morfological variability in natural populations of mat forming cyanobacteria in the Salinas of Huacho-Lima, Peru. Asch. Hydrobiol, 92, p.423-441, 1991.

MORAIS, M.G., COSTA, J.A.V. Biofixation of carbon dioxide by Spirulina sp. and Scenedesmus obliquus cultived in a three-stage serial tubular photobioreactor. J. Biotechnol., 129, p.439–445, 2007.

MORGAN, P.H., MERCER, L.P., FLODIN, N.W. General model for nutritional responses of higher organisms. Proc. Nat. Acad. Sci., 72, p.4327- 4331, 1975.

MOSER, H., The dynamics of bacterial populations maintained in the chemostat. The Carnegie institute, Washington, DC, 1958.

MOTULSKY, H.J., CHRISTOPOULOS, A. Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting. GraphPad Software Inc., San Diego CA, www.graphpad.com, 2003.

MOTULSKY, H.J., RANSNAS, L.A. Fitting curves to data using nonlinear-regression – a practical and nonmathematical review. Faseb Journal, 1; p.365–374, 1987.

MULLER-FEUGA, A., GUEDES, R., PRUVOST, J., 2003. Benefits and limitations of modeling for optimization of Porphyridium cruentum cultures in an annular photobioreactor. Journal of Biotechnology, 103, 153-163.

MUNDT, S., KREITLOW, S., NOWOTNY, A., EFFERMET, U. Biochemical and pharmacological investigations of selected cyanobacteria. Int. J. Hyg. Environ. Health 203, p.327-334, 2001.

MUÑOZ, R.; KÖLLNER, C.; GUIEYSSE, B.; MATTIASSON, B. Photosynthetically oxygenated salicylate biodegradation in a continuous stirred tank photobioreactor. Biotechnology and Bioengineering, 87, n.6, p.797-803, 2004.

MURAKAMI, T; IKENOUCHI, M. The biological fixation and utilization project by rite (2)-screening and breeding of micro algae with high capability in fixing CO_2. Energy Convers. Mgmt, 38, p. 493-497, 1997.

NAKASHIMA, S.M.K., ANDRÉ, C.D.S., FRANCO, B.D.G.M., Review: Basici Aspects of Predictive Microbiology, Brazilian Journal of Food Technology, 3, p.41-51, 2000.

NEELEMAN, R. Biomass performance: monitoring and control in bio-pharmaceutical production. Thesis Wageningen University, 2002.

NETER, J., WASSERMAN, W., KUTNER, M.H., Applied linear statistical models: regression, analysis of variance, experimental designs. 3.ed, Homewood: Richard d. Irwing, 1127, 1996.

NORONHA, M. F. R. L. S. Produção e Caracterização de Microalgas para fins de Aquacultura. Relatório de Estágio do Curso de Licenciatura em Biologia Marinha e Pescas – Universidade de Algarve, Faro, 1989.

OGBONNA, J.C., YADA, H., MASUI, H., TANAKA, H.. A Novel Internally Illuminated Stirred Tank Photobioreactor for Large-Scale Cultivation of Photosynthetic Cells. JOURNAL OF FERMENTATION AND BIOENGINEERINC, 82, n.1, p.61-67. 1996.

ONO, E., CUELLO, J. L., Design parameters of solar concentrating systems for CO2-mitigating algal photobioreactors. Energy, the International Journal, 29, p.1651–1657, 2004.

ONO, E.; CUELLO, J.L. Carbon dioxide mitigation using thermophilic cyanobacteria. Biosystems Engineering, n.96, p.129-134, 2007.

ONO, E.; CUELLO, J.L. Feasibility assessment of microalgal carbon dioxide sequestration technology with photobioreactor and solar collector. Biosystems Engineering, 95.p.597-606, 2006.

P. VAN DER STEEN, A. BRENNER AND G. ORON , An integrated duckweed and algae pond system for nitrogen removal and renovation. Water Sci Technol, **38,** p.335–343, 1998.

PÁDUA, V. L. Contribuição ao estudo da remoção de cianobactérias e microcontaminantes orgânicos por meio de técnicas de tratamento de água para consumo humano. Rio de Janeiro: ABES, 2006.

PEARL, R., REED, L.J. On the rate of growth of the population of the United States since 1790 and its mathematical representation. Proc. Nat. Acad. Sci., 6, p,275–288; 1920.

PELIZER, H.L, PONTIERI H.M, MORAES O.I. Utilização de resíduos agroindustriais em processos biotecnológicos como perspectiva de redução do impacto ambiental. São Paulo: Journal of Technology Management & Innovation, 2, 2007.

ONO, E., CUELLO, J.L. Carbon Dioxide Mitigation using Thermophilic Cyanobacteria. Biosystems Engineering,96 (1), 129–134, 2007.

PEÑA-CASTRO, J.M., MARTINEZ-JERONIMO, F., ESPARZA-GARCIA, F., CAÑIZARES-VILLANUEVA, R.O. Heavy metals renoval by the microalga Scenedesmus incrassatulus in continuous cultures. Bioresource Technology, 94, 219-222, 2004.

PERNI, S., ANDREW, P.W., SHAMA, G. Estimating the maximum growth rate from microbial growth curves: definition is everything. Food Microbiology 22, 491-495, 2005.

PETROBRAS: Relatório de desempenho de gestão de emissões atmosféricas da PETROBRAS, 2005.

PEW CENTER ON GLOBAL CLIMATE CHANGE. Acessado em 06/06/2009. Disponível em: http://www.pewclimate.org/

PRICE, G.D., BADGER, M.R., WOODGER, F.J, LONG, B.M. Review Article: Advances in understanding the cyanobacterial CO2-concentrating-mechanism (CCM): functional components, Ci transporters, diversity, genetic regulation and prospects for engineering into plants. Journal of Experimental Botany, 1-21, 2007.

PULZ, Q., GROSS, W. Valuable products from biotechnology of microalgae. Appl. Mircrobiol Biotechnol, 65, 635-648, 2004.

QUEIROZ, M.I.; ZEPKA, L. Q.; JACOB-LOPES, E. Evaluation of conditions of cultivation in profile the fattys acids of Aphanothece microscopica Nageli. 1, p.204-209. IN: International Congress Engineering and Food, 2004.

QUEIROZ, M.I.; et al. Avaliação do teor de proteína da Aphanothece sp. Caracterização em aminoácidos. IN: Anais do XVI Congresso Brasileiro de Ciências e Tecnologia de Alimentos, v.2, n 1, p. 1089-1091, 100p., 1998.

QUEIROZ, M.I.; SOUZA-SOARES, L.; KOETZ, P.R.; BARROS, F.A.; DADALT, G. BENITES, C. Avaliação da Qualidade da Proteína da Cianobactéria Aphanothece sp, visando sua utilização na dieta alimentar. XVI Congresso Brasileiro de Ciência e Tecnologia de Alimentos.v.2, p.1089,1999.

QUEIROZ, M.I.; TREPTOW, R.O., KOETZ, P.R. Caracterização sensorial do odor da Aphanothece microscopica Nägeli desidratada. Boletim do Centro de Pesquisa e Processamento de Alimentos, v.16, n.1., p-55-70, 1998.

RAVEN, P. H.; EVERT, R. F.; EICHHORN, S. E. Biologia Vegetal. 6ª Edição. Rio de Janeiro: Guanabara Koogan, 2001.

RADMER, R.J. Algal Diversity and Commercial Algal Products. American Institute of Biological Sciences. 46, 4, 263-270, 1996.

REDDY, B.S., ASSENZ, G.B. The great climate debate. Energy Policy, article in press, 2009.

RODRIGUEZ, G.V., YOUSSEF, C.B., VILANOVA, J..W. Two-step modeling of the biodegradation of phenol by an acclimated activated sludge. Chemical Engineering Journal, 117, p.245-252, 2006.

RODRIGUEZ-BUEY, M.L., ORÚS, M.I. The response of *Synechococcus* PCC7942 (Cyanophyta) to changes in CO2 supply in relation to the acclimation of the CO2 concentration mechanism. I: physiological study. Journal of Plant Physiology, 158, p.325-334, 2001.

RORRER, G.; CHENEY, D. Bioprocess engineering of cell and tissue cultures for marine seaweeds. Aquacultural Engineering, v.32, p.11–41, 2004.

ROSS, T., Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol., 81, p.501–508, 1996.

SANT'ANNA, C. L. Manual ilustrado para identificação e contagem de cianobactérias planctônicas de águas continentais brasileiras. Rio de Janeiro: Interciência, 2006.

SCHIMIDELL, W., LIMA, U.A., AQUARONE, E., BORZANI, W. Biotecnologia Industrial Volume 2, Editora Edgar Blüncher, 2005.

SCHMETTERER, G. Cyanobacterial Respiration. In: BRYANT, D.A. The Molecular Biology of Cyanobacteria. Kluwer Academic Plubishers. p.409-435, 1994.

SCHOPF, J.W. Ritmo e modo da evolução microbiana pré-cambriana. Estud. Av., 9, 23, p.195-216, 1995.

SCHUCHARDT, ULF; RIBEIRO, MARCELO L.; GONCALVES, ADILSON R.. The petrochemical industry in the next century: how to replace petroleum as raw material. Quím. Nova., São Paulo, v. 24, n. 2, 2001.

SHASHIREKHA, S., UMA, L., SUBRAMANIAN, G. Phenol degradation by the marine cyanobacterioum Phormidium valderianum BDU 30501. Journal of Industrial Microbiology & Biotechnology, 19, 130-133, 1997.

SHIVELY, J.N., ENGLISH, R.S., BAKER, S.H., CANNON, GC. Carbon cycling: the prokaryotic contribution. Current opinion in microbiology, 4, p.301-306, 2001.

SHULER, M.L., KARGI, F. Bioprocess Engineering: basic concepts. Englewood Cliffs, Prentice-Hall, 1992.

SIERRA, E., ACIÉN, F.G., FERNÁNDEZ, J.M., GARCÍA, J.L., GONZÁLEZ, C., MOLINA, E. Characterization of a flat plate photobioreactor for the production of microalgae. Chemical Engineering Journal, 138, p.136-147, 2008.

STEWART, C.; HESSAMI, M. A study of methods of carbon dioxide captureand sequestration—the sustainability of a photosynthetic bioreactor approach. Energy Conversion and Management, n.46, p.403–420, 2005.

STEWART, W.D.P.; PEMBLE, M. & UGALLY, L. Nitrogen and utilization in blue-green algal. Mitt. Internat. Limnol., v.21, p.224-247, 1978.

STRIGUL, N., DETTE, H., MELAS, V.B. A practical guide for optimal designs of experiments in the Monod model. Envirionmental Modelling & Softwater, 24, p.1019-1026, 2009.

TEISSIER, G. Le lois quantitatives de la croissance. Ann. Phisiol-Chim. Biol., 12, 527-73, 1936.

TRAVIESO, A. P., BEN YTEZ, F., SANCHEZ, E., BORJA, R., O'FARRILL, N., WEILAND, P.; Bioalga reactor: preliminary studies for heavy metals renoval; Biochemical Engineering Journal, 12, p.87-91, 2002.

TRAVIESO, L., HALL, D.O., RAO, K.K., BENÍTEZ, F., SANCHÉZ, E., BORJA, R. A helical tubular photobioreactor producing Spirulina in a semicontinuous mode. Int 413, Biodet Biodeg. 47, p.151-155, 2001.

TOMKIEWICZ, M. Global warming: science, money and self-preservation. C.R. Chimie, 9,172-179, 2006.

VAN IMPE, J.F., POSCHET, F., GEERAERD, A.J., VEREECKEN, K.M. Towards a novel class of predictive microbial growth models. International Journal of Food Microbiology, 100, 97-105, 2005.

VERHULST, P. Notice sur la population suit dans son accroissement. Corresp. Math. Phys., 10, p.113–121, 1838.

VOLTOLINA, D., CORDERO, B., NIEVES, M., SOTO, L.P. Growth of *Scenedesmus* sp. in artificial wastewater. Bioresource Technology, 68, p.265-268, 1998.

VOLTOLINA, D., GOMEZ-VILLA, H., CORREA, G. Nitrogen removal and recycling by Scenedesmus obliquus in semicontinuous cultures using artificial wastewater and a simulated light and temperature cycle; Bioresource Technology, 96, p.359-362, 2005.

WEBB, F.C. Biochemical Engineering, Van Nostrand, 1964.

WHITING, R.C., BUCHANAN, R.L. A classification of models for predictive microbiology. Food Microbiol., 10, p.175-177, 1993.

WHITING, R.C., BUCHANAN, R.L.; Predictive Modeling; In: DOYLE, M.P., BEUCHAT, L.R., MONTVILLE, T.J.; Food microbiology-fundamentals and frontiers; Washington: ASM; p.728-739, 1997.

WIJANARKO, A., GOZAN, M., ANDIKA, S.M.K., WIDIASTUTI, P., HERMANSYAH, H., WITATO, A.B., ASAMI. K., WIDANINGROEM, W., OHTAGUCHI, K, KOO, S.S. Enhancement of carbon dioxide fixation by alteration of illumination during *Chlorella vulgaris-Buetenzorg's* growth. Biotechnology and Bioprocess Engineering, 11, p.484-488, 2006.

WIJANARKO, A., OHTAGUCHI, K. Carbon dioxide removal and biomass production by Anabaena cylindrica IAM M1 using reactor in series. Studies in Surface Science and Catalysis, 153, p.461-468, 2004.

WIJANARKO, A., OHTAGUCHI, K. Elevation of the efficiency of cyanobacterial Carbon Dioxide removal by Monoethanolamine solution. Technology, 8, p.267-285, 2002.

YAGISHITA, T., SAWAYAMA, S., TSUKAHARA, K., OGI, T.; Photosynthetic Bio-Fuel Cells using cyanobacteria; WREC,1996.

YANG, H., XU, Z., FAN, M., GUPTA, R., SLIMANE, R.B., BLAND, A., WRIGHTS, I.. Progress in carbon dioxide separation and capture: A review. Journal of Environmental Sciences, 20, p.14–27, 2008.

YANO, T., KOGA, S. Dynamic behaviour of the chemostat subject to substrate inhibition. Biotechol. Bioeng., 11, 2, p.139-153, 1969.

YUN, Y.S., LEE, S.B, PARK, J.M., LEE, C., YANG, J.W. Carbon dioxide fixation by algal cultivation using wastewater nutrients. Journal of Chemical Technology and Biotechnology, 69, p.451-455; 1997.

ZEESHAN, M., PRASAD, S.M. Differential response of growth, photosynthesis, antioxidante enzymes and lipid peroxidation to UV-B radiation in tree cianobactéria. South African Journal of Botany, article in press, 2009.

ZURERA-COSANO, G., GARCÝA-GIMENO, R.M., RODRÝGUEZ-PÉREZ, R., HERVAS-MARTÝNEZ, C., Performance of response surface model for prediction of Leuconostoc mesenteroides growth parameters under different experimental conditions. Food Control, 17, p.429–438, 2006.

ZWIETERING, M.H., JONGENBURGER, I., ROMBOUTS, F.M., VAN'T RIET, K., Modeling of the bacterial growth curve. Appl. Environ. Microbiol., 56, p.1875–1881, 1990.

CAPÍTULO 2

Improving refinery wastewater for cyanobacterial biomass production and CO_2 biofixation: predictive modeling and simulation

ABSTRACT

The objective of this research was to evaluate the use of refinery wastewater in cyanobacteria cultivation for CO_2 biofixation process and biomass production. Two strategies were considered in making the use of wastewater viable to support biomass growth: (i) wastewater dilution in synthetic BGN medium, and (ii) wastewater with supplementation of salts of synthetic medium. For the best experimental condition the kinetics growth data were adjusted using five mathematical growth models (Logistic, Gompertz, Gompertz Modified, Baranyi, and Morgan) in order to define an adequate mathematical expression to describe the biomass growth and to estimate the productivity in a continuous process. The results demonstrated that the addition of 25% of the salts (w/v) in the BGN medium to wastewater favors cyanobacteria growth. Using statistical and physiological significance criteria, the Modified Gompertz model was considered the most adequate to describe biomass growth. The predictive analysis shows the possibility to obtain $1.41 kg_{biomass}/L_{reactor}$ in 1000h of continuous process with a consequent biofixation of $2.61 kg_{CO2}/L_{reactor}$ in parallel.

Keywords: cyanobacteria, predictive modeling, refinery wastewater, CO_2 biofixation.

1 Introduction

It is generally recognized that cyanobacteria play an important role in the self-purification of natural waters, since cyanobacteria use solar energy to supply oxygen required for aerobic degradation and recycle the nutrients responsible for eutrophication into potentially valuable biomass (Muñoz and Guieysse, 2006; Queiroz et al., 2007).

The use of cyanobacteria for wastewater treatment was suggested years ago (Caldwell, 1946; Oswald and Gotaas, 1957). Cyanobacteria may offer an inexpensive alternative to conventional forms of tertiary wastewater treatment. There is considerable information on the advantages of using photosynthetic organisms for the amelioration of wastewater, since photosynthesis produced oxygen is effective in BOD removal, even of refractory organics, and because the high pH values typical of dense cyanobacterial cultures cause phosphate and heavy metal precipitation. Also, cyanobacteria have a negative surface charge and, therefore, a high affinity for heavy metal ions, which makes them especially effective in wastewater detoxification (Voltolina et al., 1998).

The adaptation of using cyanobacteria in refinery wastewater is an alternative, economically speaking, because such wastewater contains considerable quantities of nitrogen, phosphorus, carbon, and micronutrients which could be reused for microbial growth (Chojnacka et al., 2004; Yun et al., 1997). The main limitation in using this wastewater is related to the composition and the possible presence of oils, solvents, high biochemical oxygen demand, suspended solids, aromatics, phenols, sulphides, halogenated and polycyclic aromatic compounds, and other compounds that can inhibit microalgal growth. Thus, the first step in promoting an efficient biomass production and CO_2 fixation system is the optimization of the culture media conditions. This can be done by testing different points of the treatment and different dilution conditions and salt supplementation (Joseph and Joseph, 2001).

Mathematical models or kinetic models can be used to improve the culture medium and to solve different types of problems, such as design, economical optimization, process synthesis, and control (Baquerisse et al., 1999). These models can be solved using curve-fitting algorithms, however, special care must be taken in determining the initial values because they can radically impact the fit of the model. A poor starting point can result in a divergent or incorrect solution (Motulsky and Ransnas,

1987). It is also important to be able to objectively quantify the goodness-of-fit of the resulting model in order to determine its utility as compared to other possible models (Yu et al., 2007).

To compare different predictive models, it is necessary to consider 5 basic criteria: (i) goodness of fit and accuracy against observed data; (ii) relative complexity (parsimony); (iii) ease of synthesis and use; (iv) potential for physiological significance; and, (v) interpretation of model parameters. These are important considerations to be made so as to avoid the challenge that a model is only an exercise in curve fitting (Phua and Davey, 2007).

After the optimization of the culture medium, another important step for the development of industrial cyanobacteria biotechnology is the maximization of biomass productivity in photobioreactors, which implies the improvement of cultivation parameters and knowledge of growth characteristics of the microorganism. Several models have been developed to predict growth and volumetric productivity in photobioreactors. Such models can provide important insight into the dependence of mass productivity on certain parameters of a photobioreactor. However, these models generate only qualitative information and cannot be extrapolated to other photobioreactor configurations, other strains, or even to different cultivation conditions. Steady-state culture characteristics are commonly used for productivity optimization and for cell physiology studies in continuous cultures, and are normally achieved by using the conventional chemostat cultivation (Barbosa et al., 2003). According to Prat et al. (2006) the theoretical study of the batch transposition to continuous process is an important step to couple technological improvement with experimental design. These authors report the possibility of initial development of the continuous process based on transient regimes analysis.

In this regard, this study aimed to evaluate the use of refinery wastewater in cyanobacteria cultivation for CO_2 biofixation process and biomass production. The focus is optimization of the culture media conditions, mathematical modeling of growth, and prediction of biomass productivity in a continuous process.

2 Materials and methods

2.1. Microorganism and culture conditions

Axenic cultures of *Aphanothece microscopica Nägeli* (RSMan92) were originally isolated from the Patos Lagoon estuary, from the state of Rio Grande do Sul, Brazil (32°01´S - 52°05´W). Stock cultures were propagated and maintained in standard synthetic BGN medium (Ripka et al., 1979) with the composition presented in Table 2.1 and pH 8.0. The incubation conditions used were 25ºC, photon flux density of 15μmol.m^{-2}.s^{-1}, and a photoperiod of 12h. For the experiments, the inoculum was acclimatized to the refinery wastewater by maintaining it for 30 days under gradual addition of the wastewater into the synthetic medium.

Table 2.1Composition of synthetic medium BGN (pH 8.0)

Parameter	Value (mg/L)	Parameter	Value (mg/L)
$CaCl_2.6H_2O$	0.04	**Cítric acid**	6.00
$CuSO_4.5H_2O$	0.079	**Na_2CO_3**	20.00
$ZnSO_4.7H_2O$	0.22	**K_2PO_4**	30.00
$Na_2MoO_4.2H_2O$	0.39	**$CaCl_2.2H_2O$**	36.00
Na_2EDTA	1.00	**NaCl**	72.00
$MnCl_2.4H_2O$	1.81	**$MgSO_4.7H_2O$**	75.00
H_3BO_3	2.86	**$NaNO_3$**	1500.00
Ammonium Iron(III) Citrate	6.00		

2.2. Development of culture medium

Refinery wastewater was utilized in the experiments. The wastewater was obtained from the Paulinia Refinery (Replan/Petrobras, São Paulo, Brazil). The wastewater was collected from the discharge point of the activated sludge treatment for 8 months from May to December of 2007, and analyzed for pH, temperature, biochemical oxygen demand (BOD), nitrite, nitrate, ammonia, phosphate, phenol, cyanide, oil and grease, and total suspended solids (TSS) following Standard Methods (APHA, 1998) and the result are presents in Table 2.2. The point of wastewater treatment used was defined in preliminary tests of three points of refinery wastewater treatment. Table 2.3 presents the composition of the mediums tested to evaluate the viability of using wastewater to promote cell growth. Two strategies were considered viable for the use of wastewater to support biomass

growth: wastewater dilution in synthetic BGN medium (volume per volume) and wastewater with salt supplementation of the same synthetic medium (weight per volume).

Table 2.2: Composition of wastewater from refinery industry

Parameter	Value*
PH	8.3 ± 0.24
Temperature (ºC)	28.1 ± 2.41
BOD (mg/L)	14.0 ± 1.36
Nitrite (mg/L)	0.1 ± 0.00
Nitrate (mg/L)	15.4 ± 0.32
Ammonia (mg/L)	1.2 ± 0.10
Phosphate (mg/L)	0.5 ± 0.00
Phenol (mg/L)	0.02 ± 0.00
Cyanide (mg/L)	0.04 ± 0.00
Oil and grease (mg/L)	4.6 ± 0.38
TSS (mg/L)	0.13 ± 0.00

*Values are means ± SD of all months considered.

Table 2.3: Composition of the culture mediums tested

Culture Medium	Composition
M1	refinery wastewater
M2	synthetic BGN medium
M3	75% wastewater and 25% BGN
M4	50% wastewater and 50% BGN
M5	25% wastewater and 75% BGN
M6	wastewater with 100% BGN salts supplementation
M7	wastewater with 75% BGN salts supplementation
M8	wastewater with 50% BGN salts supplementation
M9	wastewater with 25% BGN salts supplementation

2.3. Photobioreactor design

Measurements were made in a bubble column photobioreactor (Jacob-Lopes et al., 2008a). The system was built with 4mm thick glass, had an internal diameter of 7.5cm, a height of 75cm, and a nominal working volume of 3.0L. The dispersion system for the reactor consisted of a 1.5cm diameter air diffuser located in the centre of the column. The reactor was continuously illuminated with sixteen 40W fluorescent daylight-type tubes (General Electric, Brazil), connected in parallel and located in a photoperiod chamber. Airflow into the photobioreactor was provided via a filtered air and pure CO_2

cylinder (Praxair, Inc, Brazil) through Teflon tubing. The CO_2/air mixture was adjusted to achieve the desired concentration of carbon dioxide in the air-stream through three rotameters that measured the flow rates of the carbon dioxide, the air, and the mixture of gases, respectively. The diagram of the experimental apparatus used is shown in Figure 2.1.

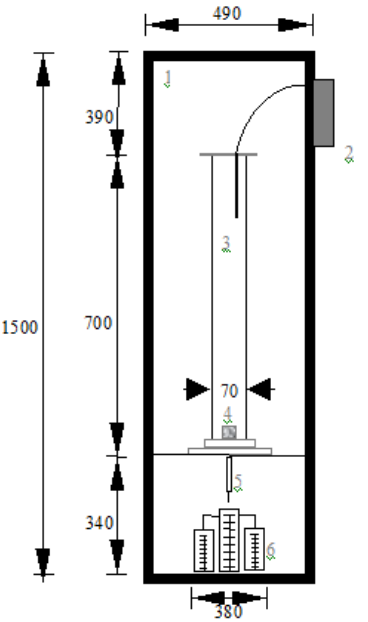

Figure 2.1: Photobioreactor diagram.

1: photoperiod chamber; 2: pH, temperature and CO_2 analyser; 3: photobioreactor; 4: gas diffuser; 5: gases inlet; 6: system controlling the flow rate and mixture of the gases (Air flow meter and CO_2 flow meter). All dimensions in mm.

2.4. Obtaining the kinetic data

The experiments were carried out in bioreactors operating in a batch mode, fed with 3.0L of different culture medium. The experimental conditions were as follows: initial cell concentration of $0.1 g.L^{-1}$, isothermal reactor operating at a temperature of 30ºC, photon flux density of $150 \mu mol.m^{-2}.s^{-1}$, and continuous aeration of 1VVM with the injection of air containing 15% carbon dioxide in volume. The cell concentration, and elemental composition of cells were monitored every 12h during the microbial growth phases. Residence times of up to 156h were considered for all the experiments. The tests were carried out in duplicate and the kinetic data referred to the mean of four repetitions.

2.5. Analytical methods

The cell concentration was evaluated gravimetrically by filtering a known volume of culture medium through a 0.45μm filter and drying at 60°C for 24 hours. The photon flux density was determined using a digital photometer (Spectronics, model XRP3000), measuring the light incident on the external reactor surface. The temperature was controlled using thermostats and measured using a polarographic probe (Mettler Toledo, lnPro5000 series). The flow rates of the carbon dioxide, air, and CO_2 enriched air were determined using rotameters (AFSG 100 Key Instruments). The composition of the elements of the *Aphanothece microscopica Nägeli* cells was determined using a Perkin Elmer 2400 CHNS/O element analyser. Two-milligram samples of biomass were oxidised at 1000ºC and the resulting gases were determined using a thermal conductivity probe for carbon. The standard used was acetanilide, with a composition of 71.09% carbon, 11.84% oxygen, 6.71% hydrogen, and 10.36% nitrogen.

2.6. Mathematical models and statistical validation

The parameters of for microbial growth curves can be obtained by non-linear regression techniques. The Logistic Function model describes the growth of microbial populations as a function of initial population density, time, growth rate, and final population density. The original Logistic Function model was developed by Pearl and Reed (1920) based on earlier insights by Verhulst (1838). On this basis the Logistic expression became:

$$y = \frac{A + C}{1 + exp^{-B(t-M)}} \quad (1)$$

A large number of growth functions have been derived from the classic model developed by Gompertz (Gompertz, 1825). The original expression is represented by Eq. 2 and the lag phase is not considered:

$$y = A + Cexp^{-exp[B(t-M)]} \quad (2)$$

The Gompertz expression was re-parameterized by Zwietering and co-workers to include three biologically relevant parameters: lag time (λ), specific growth rate (μ_{max}), and the asymptotic value or

maximum cyanobacteria population ($C=X_{max}/X$) (Zwietering et al., 1990). This model can be represented by:

$$y = C.exp^{-exp\left[\frac{\mu_{max}.exp(1)}{C}(\lambda - t)+1\right]} \quad (3)$$

The Baranyi model is geometrically different since it shows a quasi-linear segment during the exponential phase. In the model proposed by Baranyi and co-workers in 1993, the variation of the cell population (X) with time is described by a first-order differential equation. In 1994, Baranyi and Roberts derived solutions to this differential equation, under certain conditions, using 6 parameters, and in 1997, Baranyi reduced the solutions of this differential equation to four parameters (Baranyi et al., 1993; Baranyi and Roberts, 1994; Baranyi, 1997):

$$y = \mu_{max}A(t) - ln\left(1 + \frac{exp^{\mu_{max}.A(t)} - 1}{exp^{C}}\right) \quad (4)$$

Where:

$$A(t) = t + \frac{1}{\mu_{max}} ln\left(exp^{-\mu_{max}.t} + exp^{-\mu_{max}.\lambda} - exp^{-\mu_{max}.(t+\lambda)}\right) \quad (5)$$

The Morgan model is another expression usually used to describe biomass growth (Morgan et al., 1975):

$$y = \frac{A.t^{v}}{K^{v} + t^{v}} \quad (6)$$

The Newton Method was used to solve these nonlinear equations (1-6) in Microsoft Excel® XP Software. Initial estimates of the parameters values were required and a good choice of initial values is critical since a poor starting point can result in divergence or a wrong solution (Motulsky and Ransnas, 1987). A reasonable initial estimate for the coefficient was obtained through the graph (ln X) vs. (t).

Least Square was used in Microsoft Excel® XP Software to solve the models through an iterative procedure which determines the minimization of the least value of the square error sums between the empirical and predicted values.

Indices of performance of the predictive models were calculated by the following mathematical and statistical expressions (Ross, 1996):

Root mean square error (RMSE):
$$RMSE = \sqrt{\frac{\sum (obs - pred)^2}{n}} \quad (7)$$

Standard error of prediction (%SEP):
$$\%SEP = \frac{100}{mean\ obs}\sqrt{\frac{\sum (obs - pred)^2}{n}} \quad (8)$$

Bias factor (Bf):
$$B_f = 10^{\frac{\sum log(pred/obs)}{n}} \quad (9)$$

Accuracy factor (Af):
$$A_f = 10^{\frac{\sum |log(pred/obs)|}{n}} \quad (10)$$

2.7. Optimizing the operation conditions

In a continuous photobioreactor, the operation often starts with a short batch period intended to increase cell concentration prior to dilution. The best duration for this preliminary batch period has been considered. So, to provide a theoretical answer to the question of how biomass production can be optimized, the growth model (previously defined) and mass balance to CSTR operation were considered. Mass balance for biomass in a continuous culture mode can be represented by (Muller-Feuga et al., 2003):

$$X_t = X_0 exp^{(\mu - D)t} \quad (11)$$

3 Results and Discussions

3.1. Optimization of the culture medium

To evaluate the performance of the cyanobacteria cultivated in refinery wastewater, a comparison between cultivations of refinery wastewater (M1) and synthetic BGN medium (M2) was initially made. Figure 2.2 shows the growth data obtained from these experiments. The analysis of kinetic growth data indicates a reduced cellular adaptation in the wastewater when compared with the cultivation in the synthetic medium. Maximum cellular concentrations of 0.14g/L were obtained in 48h of residence time in the M1 culture medium, while 5.10g/L were reached in 156h in the M2 culture medium. The values obtained for cultivation in wastewater represent less than 3% of the maximum cellular densities obtained in the BGN medium cultivation, suggesting the presence of inhibitor compounds of cellular growth, associated with the limitation of essential nutrients to cyanobacteria growth in wastewater. These results can be corroborated by analyzing Table 2.2, which presents the chemical composition characterization of the refinery wastewater used. An analysis of the data shows the presence of inhibitors of growth substances, for example, phenolic compounds and cyanide, low concentrations of nitrogen and phosphorous, and also the lack of micronutrients essential for microbial development when compared with the synthetic BGN medium (Hodaifa et al., 2008; Joseph and Joseph, 2001). Isolated or combined, these characteristics can explain the reduced performance of the microbial growth in refinery wastewater. Figure 2.3 shows the growth data obtained from the others experiments.

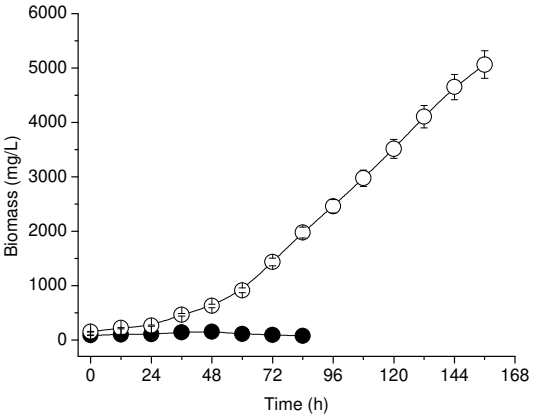

Figure 2.2: Growth curves in the refinery wastewater (closed symbols) and in thesynthetic BGN medium (open symbols)

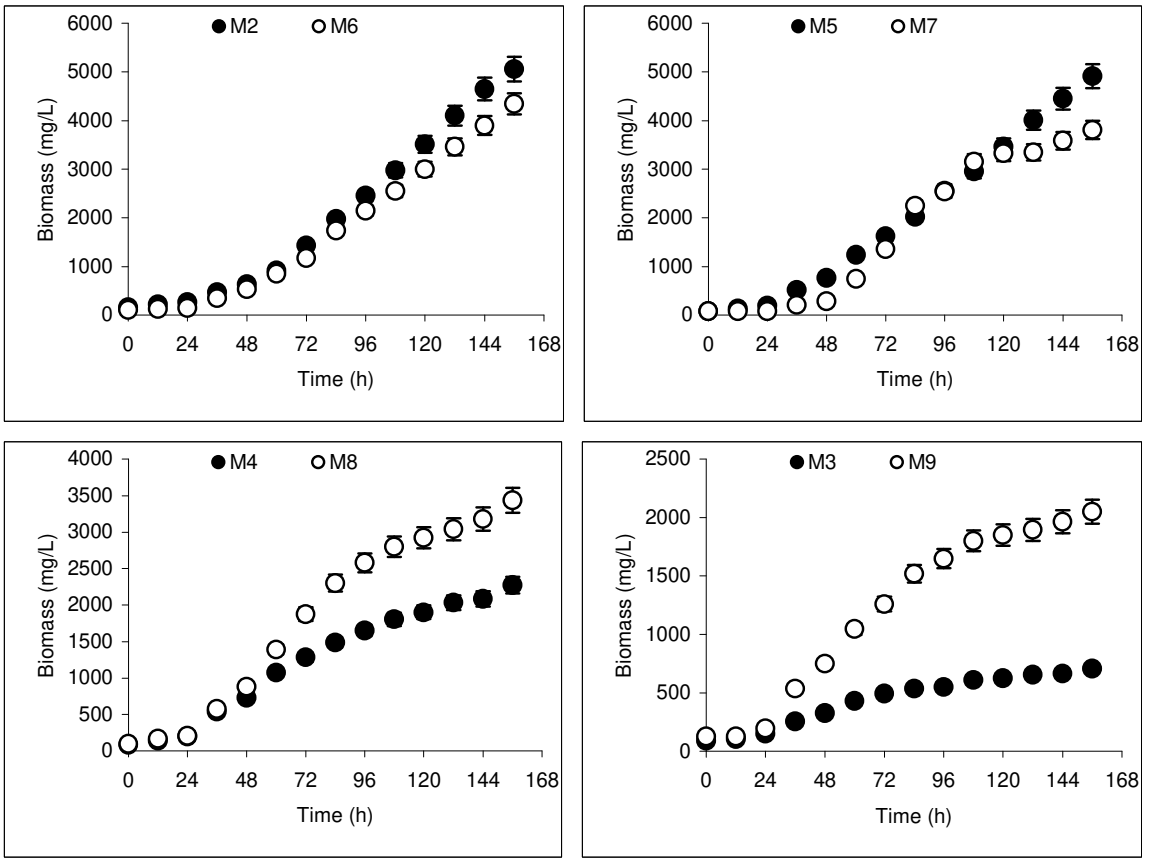

Figure 2.3: Growth curves of the experiments

To enable the effective use of refinery wastewater to support biomass growth, wastewater dilution in synthetic medium and the supplementation of salts in the wastewater was considered. Table 2.4 presents the growth data of *Aphanothece microscopica Nägeli* on different cultivation mediums tested. The analysis of kinetic parameters suggests the reduction of maximum cellular densities (X_{max}) with an increase of wastewater concentration in the culture medium, considering the wastewater dilution in the synthetic medium (Mediums M1-M5). On the other hand, the wastewater supplementation with BGN salts improved the biomass production (Mediums M6-M9).

The presence of inhibitor or toxic compounds was evaluated through the comparison between the experiments M2 (5.1g/L) and M6 (4.34g/L), which show that the maximum cell concentration was 14.2% less in wastewater with 100% BGN salts supplementation (M6). Similarly, the lack of nutrients

was verified in the wastewater cultivation with different salts supplementation, where the increase of cell concentration with the increase of the compounds added (increase of up to 96.8% in the X_{max}, considering the culture mediums M1 and M6) can be observed. These results suggest a higher magnitude of lack of essential nutrients compared to the presence of inhibitory compounds in cell growth.

Table 2.4: Growth data of *Aphanothece microscopica Nägeli* in different tests

Culture Medium	X_{max} * (g/L)	μ_{max} (d^{-1})
M1	$0.14^{a} \pm 0.00$	$0.32^{a} \pm 0.00$
M2	$5.10^{b} \pm 0.10$	$0.69^{b} \pm 0.03$
M3	$0.71^{c} \pm 0.03$	$0.62^{b} \pm 0.05$
M4	$2.27^{d} \pm 0.11$	$0.96^{c} \pm 0.06$
M5	$4.91^{e} \pm 0.24$	$1.07^{c} \pm 0.05$
M6	$4.34^{f} \pm 0.21$	$0.81^{b} \pm 0.02$
M7	$3.81^{g} \pm 0.19$	$1.24^{d} \pm 0.09$
M8	$3.42^{b} \pm 0.15$	$1.13^{d} \pm 0.06$
M9	$2.05^{h} \pm 0.09$	$1.12^{d} \pm 0.04$

* end of experiment (156h).

**Within the same column, means having different superscripts are significantly different ($p<0.05$) by Tukey test

The cells changed from a blue-green color to yellow on the second day of the cultivations in refinery wastewater (M1). This phenomenon is known as chlorosis, or bleaching, and results from the changes of environmental conditions, such as the deprivation of essential nutrients. Chlorosis is normally caused by a lack of the nitrogen and phosphorous necessary for photosynthesis (Pavlostathis & Jackson, 2002).

Differences between M2 and M6 (X_{max} and μ_{max}) can be explained as a function of stress on the cell growth. The functioning of some proteins is inhibited or lost and that of others are enhanced or induced in stress conditions. The genome and proteome of several cyanobacteria, during normal growth or acclimation to different stresses, need be analyzed in deep (Genter, 1996). The compounds inhibitor to microalgae include different chemical species of metals and also nonmetallic compounds like ozone and cyanide (Castielli et al., 2009). All elements and compounds are potentially inhibitor or toxic, but

an interesting characteristic of some metals is that they are nutrients at low concentrations (e.g., Cu, Mo, Fe, and Zn) (Genter, 1996).

The specific growth rate, μ, is a measure of how quickly a microbial population is growing. High values of μ are indicative of high microbial growth rates. This kinetic parameter determines the maintenance of the steady state in biological reactors operated at the constant volume. The values of maximum specific growth rates (μ_{max}), obtained from the graph of (ln X) vs. t, show that the use of mediums M5 ($1.07d^{-1}$) or M4 ($0.96d^{-1}$) improved the cell growth rate, since the maximum specific growth rates obtained were superior to those reached in the synthetic BGN medium (M2) ($0.69d^{-1}$), considering the experiments with dilution of the wastewater. Additionally, in the culture mediums supplemented with salts, an optimal composition of 75% salts supplementation M7 ($1.24d^{-1}$), followed by 50% of salts addition (M8) ($1.13d^{-1}$), and 25% (M9) ($1.12d^{-1}$) can be observed. A comparison between M7, M8, and M9 culture mediums showed that these cultivations are statistically equal to the Tukey test ($p<0.05$), promoting the utilization of M9 because in such conditions there is a minor requirement of salts and water added. The maximum growth rates obtained were higher than those observed by Joseph and Joseph (2001) and Chojnacka et al., (2004) when cultivating the cyanobacteria *Spirulina sp*. and *Oscillatoria quadripunctulata* in refinery wastewater, respectively, which suggests the adaptation of the microorganism and appropriateness of the methodology used. The highest maximum specific growth rate obtained with M6-M9 tests compared with M2 medium maybe can be explained with a result of modifications in metabolic pathway by stress conditions (lack of nutrients or chemicals presents in wastewater).

The pH range observed in the all culture medium varied from 8.0 to 9.3, indicating that the specie HCO_3^- was the predominant form of carbon in the cultures. The gradual increase in pH of the culture media, independent of the conditions, indicated that cultivation occurred in an alkaline pH range. According to Arnon (1984) and Zuber (1986), the increase in pH in photosynthetic cultures is an indication of the consumption of inorganic carbon due to cell growth. These authors reported that the increment in pH in the culture medium could be attributed to two main mechanisms: first, the transport of hydroxide ions to outside the cell occurs by way of a reaction catalysed by the enzyme carbon anhydrase during the conversion of bicarbonate ions inside the cell to provide CO_2 for the photosynthetic reaction, raising the pH of the culture medium. A second potential mechanism would be

the increase in pH due to activity of the enzyme ribulose 1,5-bisphosphate carboxylase, whose activity is considerably dependent on pH, increasing with an increase in pH. This enzyme is present in the photosynthetic apparatus of the cyanobacteria, where the H^+ ions are sequestered to the inside of the tylacoid membrane with a simultaneous transfer of Mg^{2+} to the environment. These light energy induced fluxes result in an increase in pH and in the Mg^{2+} concentration, activating the rubisco enzyme and resulting in efficient carbon dioxide fixation. In addition, the presence of ammonia in wastewater leads to a sharp rise of pH values in the initial periods of cultivation. This fact is more accentuated in conditions with high refinery wastewater concentrations. The precipitation of carbon dioxide by washing with alkaline solutions is one of the gas absorption processes most widely used in the chemical industry. These processes consist of capturing the CO_2 in alkaline solutions by chemical reactions of the system CO_2-H_2O-OH-. Thus, sequestering CO_2 by way of the formation of carbonates and bicarbonates in photobioreactors which use this type of wastewater could present an advantage in the improving of the global CO_2 sequestration rates, with which biological and chemical mechanisms are involved in the rise of pH values (Lee et al., 2006).

On the other hand, the presence of organic carbon in the wastewater, which could be assimilated by the cells through heterotrophic metabolism during the night periods, in the case of cultivations in photoperiod conditions (day/night cycles) should be considered. Although this condition improves the biomass production performance, the global carbon dioxide sequestration rates are strongly affected by the mixotrophic metabolism, since at night periods CO_2 is released and O_2 is consumed through heterotrophic metabolism and the contrary occurs during day periods. This consideration has a practical importance since there is large interest in operating such systems using solar energy, especially in locations near the equator where the day and night periods are very similar (Jacob-Lopes et al., 2008b).

3.2. Predictive modeling and simulation

Mathematical modeling has been widely used to predict cellular growth through the estimate of maximum specific growth rate, maximum cell concentrations, and duration of lag phase, which are all needed in the study of microbial growth and to use in industrial microbiology. Figure 2.4 presents the fit of the mathematical models to the optimized condition (M9). The visual analysis of data show an

unsatisfactory fit of the Logistic and Baranyi models to experimental data. The Logistic model does not consider the lag phase and the Baranyi model presents this criteria, but does not describe with accuracy the data obtained for the *Aphanothece microscopica Nägeli*. Gompertz, Modified Gompertz, and Morgan models present a good relation with the experimental data, so, the goodness-of-fit or reliability of growth models need mathematical evaluation before being put into practice (Dong et al., 2007). Table 2.5 presents the indices of performance of the models tested.

The RMSE provides a measure of the concordance between experimental data and the model. The best model adjust will present a reduced value of RMSE. The minor values were observed on the Modified Gompertz and Morgan models (0.11 and 0.09 respectively). The Bias factor (Bf) gives an objective indication of better performance of the model. Ross (1996) suggested that perfect agreement between predictions and observations are represented by Bf of 1. Higher or lower values indicate a systematic over or under estimation of the observed values, respectively. Models describing growth rate with Bf in the range of 0.9–1.05 could be considered good, in the range of 0.7–0.9 or 1.06–1.15 considered acceptable, and <0.7 or >1.15 considered unacceptable. Again, the calculated values show that Morgan and Modified Gompertz models (0.95 and 1.01, respectively) were the better fit. The accuracy factor represents the difference between the mean observed values and the predict values. The increase of this factor results in a low capacity for prediction of correctness between the estimated and true values. The values presented by the Morgan and Modified Gompertz were closer to 1 (1.07 and 1.06, respectively). The standard error of prediction (%SEP) confirms the reduced residuals of the Morgan and Modified Gompertz model.

Statistical analysis of the models shows that the Modified Gompertz and Morgan models were the best expressions to describe growth data of *Aphanothece*. Thus, it was necessary to evaluate the characteristics of each model to select the most appropriate to the prediction. The selection criterion was the model parameters meaning. Modified Gompertz presents parameters with physical meaning (μ_{max}, λ and X_{max}) and the Morgan model is related to the fit of curve and can't be physically interpreted. The Modified Gompertz model describes the growth data, both in terms of statistical accuracy and ease of use, when compared to other sigmoid functions (McDonald and Sun, 1999). In this sense, the Modified Gompertz was selected to predict the growth of *Aphanothece microscopica*

Nägeli and was considered statistically sufficient and robust to describe the growth data of the tested organism.

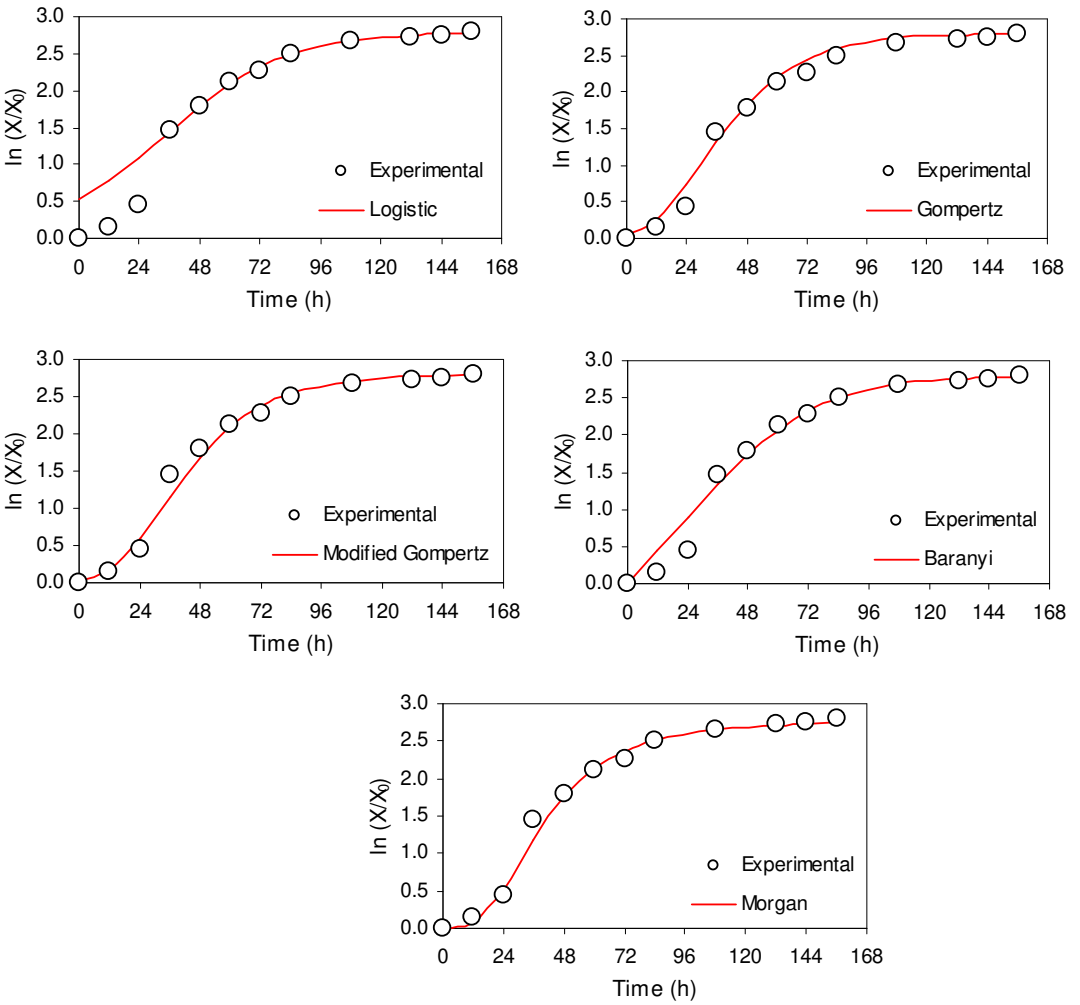

Figure 2.4: Fit of the models to experimental data

Table 2.5: Data of statistic validation models

Error Model	Gompertz	Modified Gompertz	Logistic	Baranyi	Morgan
RMSE	0.12	0.11	0.30	0.16	0.09
B_f	1.06	1.01	1.24	1.15	0.95
A_f	1.08	1.06	1.25	1.18	1.07
%SEP	6.76	5.15	16.86	8.99	4.94

The Modified Gompertz model gives the following values of the growth parameters for the M9 culture medium: μ_{max}=1.22d^{-1}, λ=15h, and X_{max}=2.05g/L. To confirm that the M9 condition is the best culture medium, the Modified Gompertz model was applied to all tested conditions (M1 – M9) to calculate μ_{max}, λ and X_{max}. A difference of 7.1% between the values of μ_{max} calculated with the mathematical growth model and the obtained from the graph of (ln X) vs. (t) was observed. Tukey test was used to analyse the statistical difference between M1 – M9 (μ_{max} obtained with Modified Gompertz). A comparison between showed that the M7, M8 and M9 cultivations are statistically equal to the Tukey test ($p<0.05$), promoting the utilization of M9 because in such conditions there is a minor requirement of salts and water added, reaffirm the utilization of M9.

After the determination of the best model to predict biomass production and growth parameters, prediction tests to evaluate the process in continuous operation were carried out. The chemostat is a rare example of a class of biological systems that has real experimental and industrial applications. In the chemostat, the most important thing to know is the batch period effects on the productivity process, because the initial time presents a batch operation period. To consider the best duration of this preliminary batch period, intending to increase the cellular concentration in the photobioreactor, the Modified Gompertz model was used to predict the biomass growth and Eq.11 to describe the cellular growth in a continuous operation. Cellular concentration was predicted in a photobioreactor as function of the dilution rate, total operation period (TOP), and batch operation period (BOP). The purpose of this study was to evaluate the magnitude of productivity gain in function of these parameters.

The predictions were carried out in the culture medium conditions previously optimized: dilution rate of $D=1.20\mu_{max}$, $D=1.10\mu_{max}$, $D=1.00\mu_{max}$, $D=0.95\mu_{max}$, $D=0.90\mu_{max}$, $D=0.85\mu_{max}$ and different BOP (18, 24, 30, 36, 42, 48, 54, 60h). These different BOP were chosen because the exponential phase began at 15h and finished at 60h for this experiment. This period was determined corroborating the data obtained between the Modified Gompertz model and the graphic determination of μ_{max}. The influence of BOP in the biomass production is expressed in Figure 2.5.

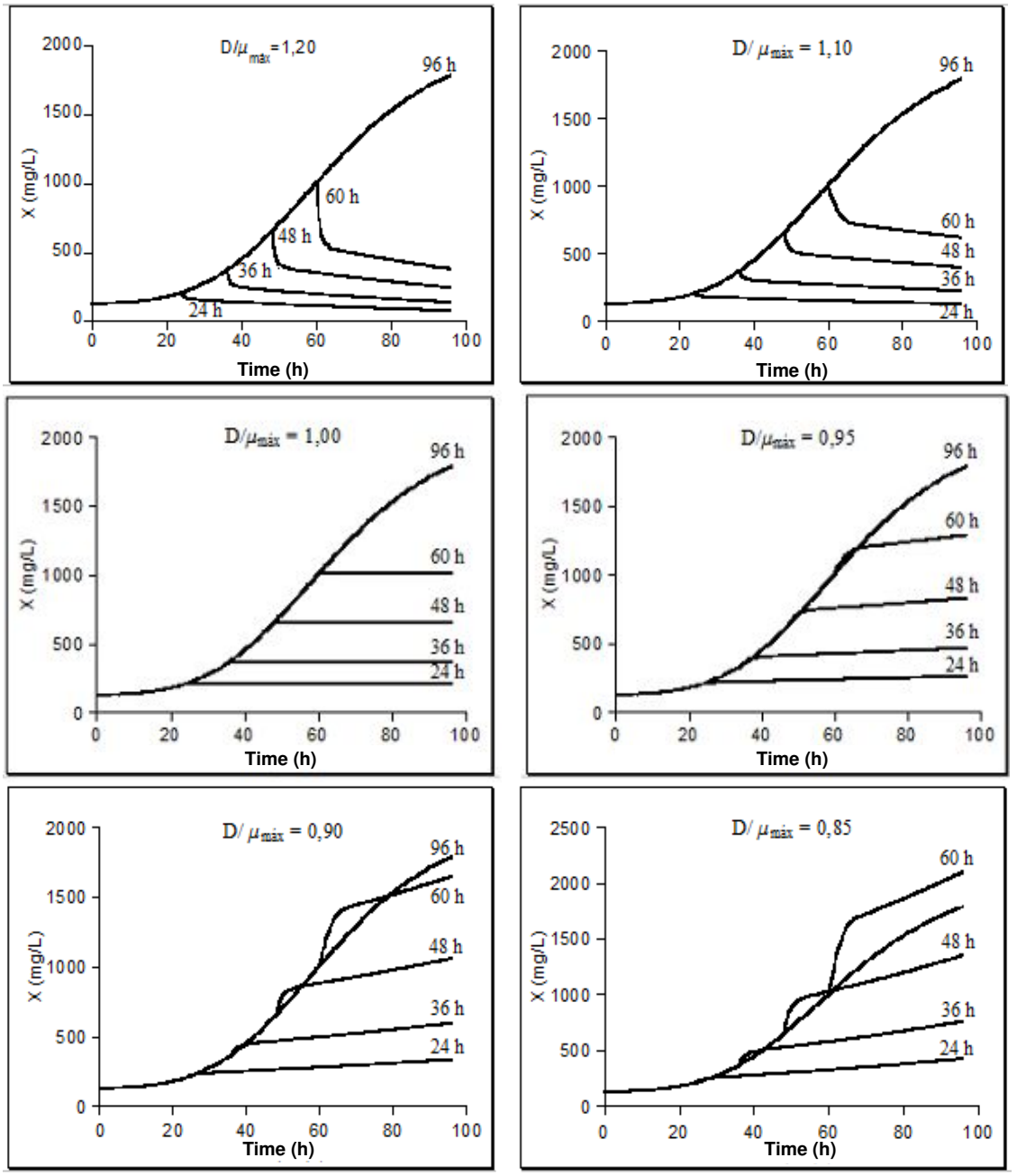

Figure 2.5: Theoretical influence of batch operation period in the biomass production.

Analysis of the curves shows the increase of biomass in function of the increase in BOP. Thus, estimating the TOP of chemostat in 1000h, it is possible to predict the magnitude of the biomass yield (Table 2.6). A simple theoretical estimate shows that it is possible to obtain an increase of 347% in the

biomass production using D=0.85μ_{max} and, consequently, CO_2 biofixation, starting the continuous process at the end of the logarithmic growth phase. In this case, the batch operation period represents 6% of the total operation period. Because of cell stability, batch operation periods of 5.4% can be considered (increase of 283%). This estimate does not consider reductions in cell growth during the process still caused by the reduction of light intensity inside the reactor, the concentration of metabolites in the culture medium, among other factors that may reduce or hinder the growth cell.

Table 2.6: Results of prediction in 1000h of continuous operation

BOP (h)	BOP/TOP (%)	Biomass (kg/L)	Increase biomass (%)
18	1.8	0.32	0
24	2.4	0.38	19
30	3.0	0.48	50
36	3.6	0.63	94
42	4.2	0.81	151
48	4.8	1.02	215
54	5.4	1.24	283
60	6.0	1.41	347

The elemental analysis of the microalgal cells at the end of cultivation showed that 1g of biomass contains 0.51±0.01g of carbon. Thus, for each 0.51$g_{biomass}$, 1.87g_{CO2} are biofixed into biomass, ie, 1.41kgBiomass/Lreactor, whit 2.61kg_{CO2} biofixed into biomass. The carbon fixed in the cells only represents a small fraction of the total CO_2 transformed by the system. Results of previous studies (Jacob-Lopes et al., 2008c) clearly indicate the existence of other routes of CO_2 conversion in the photobioreactor as precipitation of carbonates and bicarbonates, secretion of biopolymers into the culture media, and the release of volatile organic compounds (VOCs) that substantially increase the global CO_2 sequestration rates in such systems.

Therefore, the results obtained in this study indicate the potentiality of applying refinery wastewater as a partial source of nutrients and water to support cell growth and promote CO_2 biofixation. The integration of the process with suitable refinery waste gas streams as a source of CO_2, would represent an important strategy aiming to develop an economical and sustainable system to reduce greenhouse gases in oil refineries. In addition, the biomass formed has a great potentiality for biodiesel production (Zepka et al., 2008). According to Gressel (2008), cyanobacterial biomass can be

used in the manufacturing of third generation biofuels, and if the production of this biomass could be scaled up industrially, less than 6 million hectares would be necessary worldwide to meet current fuel demands, amounting to less than 0.4% of arable land, an achievable goal for global agriculture.

4 Conclusions

The presence of inhibitor compounds of cellular growth associated with the limitation of essentials nutrients to cyanobacteria growth was evident in the wastewater used. Supplementation of wastewater with 25% (w/v) BGN salts was an adequate strategy for use as a partial source of nutrients and water to support cell growth and promote CO_2 biofixation. Under these operational conditions, the microorganism performed better and maximum specific growth rates of $1.12 d^{-1}$ were obtained. The Modified Gompertz model was found to be sufficient to describe the growth of Aphanothece microscopica Nägeli, as evidenced by the statistical criteria. Despite the simplifications made, this validates the fast development method proposed to help batch to continuous transposition. The predictions indicate the possibility to obtain $1.41 kg_{biomass}/L_{reactor}$ in 1000h of continuous process with a biofixation of $2.61 kg_{CO2}/L_{reactor}$ in parallel.

5 Notation

Acronyms

BOP	Batch operation period
FBB	Full batch
TOP	Total operation period
Obs	Observed values of growth
Mean obs	Mean of observed values
Pred	Predicted values of growth parameters by kinetic models

Notations

A	Asymptotic ln count as t decreases indefinitely	-
A(t)	Precise integral of the adjustment factor	h
B	Relative growth rate at time M	h^{-1}
C	Asymptotic ln of growth that occurs as t increases indefinitely	-
K	Time when half maximal growth is achieved	h^{-1}
M	Time at which absolute growth rate at maximum	h
T	Residence time	h
X_0	Initial cell concentration	mgL^{-1}
X_{max}	Maximum cell concentration	mgL^{-1}
X_t	Cell concentration in time t	mgL^{-1}
Y	ln (X_t/X_0)	-

Greek Symbols

λ	Lag phase duration	h^{-1}
μ_{max}	Maximum-specific growth rate	d^{-1}
^{v}	Shape or curvature parameter	-

6 References

APHA, 1998. Standard Methods for the Examination of Water and Wastewater., American Public Health Association, 20º ed., D.C.

ARNON, D.I., 1984. The discovery of photosynthetic phosphorylation. Trends Biochemical Science, 9, 258-262.

BAQUERISSE, D., NOUALS, S., ISAMBERT, A., SANTOS, P., DURAND, G., 1999. Modeling of a continuous pilot photobioreactor for microalgae production. Journal of Biotechnology, 70, 335-342.

BARANYI, J., 1997. Simple is good as long as it is enough. Food Microbiology, 14, 189–192.

BARANYI, J., ROBERTS, T.A., 1994. A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology, 23, 277–294.

BARANYI, J., ROBERTS, T.A., MCCLURE, P., 1993. A non-autonomous differential equation to model bacterial growth. Food Microbiology, 10, 43–59.

BARBOSA, M.J., HOOGAKKER, J., WIJFFELS, R.H., 2003. Optimization of cultivation parameters in photobioreactors for microalgae cultivation using the A-stat technique. Biomolecular Engineering, 20, 115-123.

CALDWELL, D.H., 1946. Sewage oxidation ponds: performance, operation and design. Sewage Works International, 18, 433-458.

CASTIELLI, O., CERDA, B., NAVARRO, J.A., HERVÁS, M., ROSA, M. Proteomic analyses of the response of cyanobacteria to different stress conditions - Minireview. FEBS Letters 583 (2009) 1753–1758.

CHOJNACKA, K., CHOJNACKI, A., GÓRECKA, H., 2004. Trace element removal by Spirulina sp. from copper smelter and refinery effluents. Hydrometallurgy, 73, 147-153.

DONG, Q., TU, K., GUO, L., LI, H., ZHAO, Y., 2007. Response surface model for prediction of growth parameters from spores of Clostridium sporogenes under different experimental conditions. Food Microbiology, 24, 624-632.

GENTER, R.B. Ecotoxicology of Inorganic Chemical Stress to Algae. Algal Ecology, 1996.

GOMPERTZ, B., 1985. On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. Philos. Trans. R. Soc. London, 115, 513–585.

GRESSEL, J., 2008. Transgenics are imperative for biofuel crops. Plant Science, 174, 246–263.

HERVÁS, C., ZURERA, G., GARCÍA, R.M., MARTÝNEZ, J., 2001. Optimization of computational neural network for its application to the prediction of microbial growth in foods. Food Science Technology International, 7, 159–163.

HODAIFA, G., MARTYNEZ, M. E., SANCHEZ, S., 2008. Use of industrial wastewater from olive-oil extraction for biomass production of Scenedesmus obliquus. Bioresource Technology, 99, 1111-1117.

JACOB-LOPES, E., LACERDA, L.M.C.F., FRANCO, T.T., 2008a. Biomass production and carbon dioxide fixation by Aphanothece microscopica Nägeli in a bubble column photobioreactor, Biochemical Engineering Journal, 40, 27-34.

JACOB-LOPES, E., SCOPARO, C.H.G., FRANCO, T.T. 2008b. Rates of CO2 removal by Aphanothece microscopica Nägeli in tubular photobioreactors. Chemical Engineering and Processing, 47 1365 1373.

JACOB-LOPES, E., SCOPARO, C.H.G., LACERDA, L.M.C.F., FRANCO, T.T., 2008c. Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactors. Chemical Engineering and Processing, v.48, p. 306-310, 2009.

JOSEPH, V., JOSEPH, A., 2001. Microalgae in petrochemical effluent: growth and biosorption of total dissolved solids. Environmental Contamination and Toxicology, 66, 522-527.

LEE, B.D., APEL, W.A., WALTON, M.R., 2006. Calcium carbonate formation by Synechococcus sp. strain PCC 8806 and Synechococcus sp. strain PCC 8807. Bioresource Technology, 97, 2427-2434.

MCDONALD, K., SUN, D.W., 1999. Predictive food microbiology for the meat industry. International Journal of Food Microbiology, 52; 1-27.

MORGAN, P.H., MERCER, L.P., FLODIN, N.W.,1975. General model for nutritional responses of higher organisms. Proc. Nat. Acad. Sci. 72, 4327- 4331.

MOTULSKY, H.J., RANSNAS, L.A., 1987. Fitting curves to data using nonlinear-regression – a practical and nonmathematical review; Faseb Journal, 1, 365–374.

MULLER-FEUGA, A., GUEDES, R., PRUVOST, J., 2003. Benefits and limitations of modeling for optimization of Porphyridium cruentum cultures in an annular photobioreactor. Journal of Biotechnology, 103, 153-163.

MUÑOZ, R., GUIEYSSE, B., 2006. Algal-bacterial processes for the treatment of hazardous contaminants: A review. Water Research, 40, 2799-2815.

OSWALD, W.J., GOTAAS, H.B., 1957. Photosynthesis in sewage treatment. Trans. Am. Sot. Civ. Eng. 122, 73-105.

PAVLOSTATHIS, S.G.; JACKSON, G.H., 2002. Biotransformation of 2,4,6 trinitrotoluene in a continuous-flow Anabaena sp. System. Water Research, 36, 1699–1706.

PEARL, R., REED, L.J., 1920. On the rate of growth of the population of the United States since 1790 and its mathematical representation. Proc. Nat. Acad. Sci., 6, 275–288.

PHUA, S.T.G., DAVEY, K.R., 2007. Predictive modeling of high pressure (<700MPa)–cold pasteurization (<25°C) of Escherichia coli, Yersinia enterocolitica and Listeria monocytogenes in three liquid foods. Chemical Engineering and Processing, 46, 458–464.

PRAT, L., WONGKITTIPONG, G., ANGELOV, G., GOURDON, C., DAMRONGLERD, S., 2006. Fast batch to continuous transposition: application to the extraction of andrographolide from plants. Chemical Engineering Technology, 29(3), 401-407.

QUEIROZ, M.I., JACOB-LOPES, E., ZEPKA, L.Q., BASTOS, R.G., GOLDBECK, R., 2007. The kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor. Bioresource Technology, 98, 2163–2169.

RIPPKA, R., DERUELLES, J., WATERBURY, J.B., HERDMAN, M., STANIER, R.Y., 1979. Generic assignments strain histories and properties of pure cultures of cyanobacteria, J. Gen. Microbiol., 111, 1-61.

ROSS, T., 1996. Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol., 81, 501–508.

VERHULST, P., 1838. Notice sur la population suit dans son accroissement. Corresp. Math. Phys., 10, 113–121.

VOLTOLINA, D., CORDERO, B., NIEVES, M., SOTO, L.P., 1998. Growth of Scenedesmus sp. in artificial wastewater. Bioresource Technology, 68, 265-268.

YU, S., CLARK, O.G., LEONARD, J.J., 2008. A statistical method for the analysis of nonlinear temperature time series from compost. Bioresource Technology, 99, 1886–1895.

YUN, Y.S., LEE, S.B, PARK, J.M., LEE, C., YANG, J.W., 1997. Carbon dioxide fixation by algal cultivation using wastewater nutrients. Journal of Chemical Technology and Biotechnology, 69, 451-455.

ZEPKA, L.Q.; JACOB-LOPES, E.; GOLDBECK, R.; QUEIROZ, M.I., 2008. Production and biochemical profile of the microalgae Aphanothece microscopica Nägeli submitted to different drying conditions. Chemical Engineering and Processing, 47, 1305-1310.

ZUBER, H., 1986. Structure of light-harvesting antenna complexes of photosynthetic bacteria, cyanobacteria and red algae. Trends Biochemical Science, 11, 414-419.

ZWIETERING, M.H., JONGENBURGER, I., ROMBOUTS, F.M., VAN'T RIET, K., 1990. Modeling of the bacterial growth curve. Appl. Environ. Microbiol., 56, 1875–1881.

CAPÍTULO 3

Carbon Dioxide consumption mechanism modeling in a continuous photobioreactor using cyanobacteria

ABSTRACT

Reduce carbon dioxide in the atmosphere is a vital task in view of the climatic changes resulting from global warming caused mainly by CO_2 greenhouse gas. There are systems available for capturing CO_2, however the choice is limited, therefore this study included the evaluation of methods which are still under development. The objective of the study was to evaluate the use of models to predict CO_2 biofixation and biomass production in photobioreactors operating with cyanobacteria. For optimal experimental conditions, the kinetics substrate consumption data were adjusted using six mathematical models (Webb, Aiba, Yano & Koga, Ierusalinsky, Chen and Andrews) in order to define an adequate mathematical expression to describe the carbon dioxide consumption and to estimate the best conditions for a continuous process. Using statistical and physiological significance criteria, the results demonstrated that the Andrews model was considered the most adequate to describe carbon dioxide consumption and the predictive analysis shows that it is possible to obtain $3.00 g_{biomassa}.L^{-1}.h^{-1}$ and the maximum TRC of 2.67 $g_{CO2}L^{-1}.h^{-1}$ and the potential use of this system in processes of biological dioxide carbon fixation was demonstrated.

Keywords: cyanobacteria, predictive modeling, CO_2 biofixation, global warming.

1 Introduction

Currently one of the most pressing and globally recognized challenges we face is how to mitigate the effects of global environment change brought about by increasing emissions of greenhouse gases, especially CO_2. Several environmental policies have been proposed to deal with this problem (Woodward et al., 2009). The Kyoto protocol was based on the aim of reducing greenhouse gas emissions, especially at lowering the amount of carbon dioxide released (Hsueh et al., 2009). The most obvious way in which CO_2 emissions can be reduced is by switching from burning fossil fuels to using non-fossil-fuel sources of energy such as nuclear energy, wave and wind power, and geothermal sources (Woodward et al., 2009). Various CO_2 mitigation strategies have been investigated and there are three options to reduce total CO_2 emission into the atmosphere, i.e., to reduce energy intensity, to reduce carbon intensity, and to enhance the sequestration of CO_2. The first option requires efficient use of energy. The second option requires switching to using non-fossil fuels such as hydrogen and renewable energy. The third option involves the development of technologies to capture and sequester more CO_2 (Yang et al., 2008). This last option can be divided in three strategies: Physical, chemical and biological treatments. Biological CO_2 mitigation was carried out by plants and photosynthetic microorganisms. However, because the slow growth rates of conventional terrestrial plants, the potential for increased CO_2 capture in agriculture by plants has been estimated to contribute only 3–6% of fossil fuel emissions. On the other hand, microalgae, a group of fast-growing unicellular or simple multicellular microorganisms, have the ability to fix CO_2 while capturing solar energy with an efficiency 10 to 50 times greater than that of terrestrial plants (Wang et al., 008).

Microalgae can be divided into two types of cell structure: prokaryotic structure (*Cyanophyta-cyanobacteria* and *Prochlorophytes*) and eukaryotic structure (*Chlorophyta, Euglenophyta, Rhodophyta, haptophytes-Prymnesiophyta, Heterokontophyta* and *Cryptophyta Dinophyta*) (Derner et al., 2006). Cyanobacteria are one of the most ancient extant forms of life on Earth (Schopf et al., 1965) and the most widely distributed micro-organisms in the biosphere and they play a dominant role in the global nitrogen and carbon cycles (Schwarz and Forchhammer, 2005).

Culture of cyanobacteria in open ponds and raceways is well developed but only a few species can be maintained in traditional open systems that control contamination by using highly alkaline or saline selective environments. Fully closed photobioreactors provide opportunities for monoseptic culture of a greater variety of algae than is possible in open systems. Tubular photobioreactors that

circulate the culture by using an airlift device are especially attractive for several reasons: circulation is achieved without moving parts and this provides a robust culture system with a reduced potential for contamination; the cell damage associated with mechanical pumping is avoided; and the airlift device combines the function of a pump and a gas exchanger that removes the oxygen produced by photosynthesis (Molina et al., 1999).

Carbon dioxide mass transfer is a key factor in cultivating photosynthetic microorganisms in photobioreactors due to the low mass transfer coefficient between carbon dioxide and water (Hsueh et al., 2009). The specific growth rate, saturation constant and inhibition constant are very important parameters in biotechnological cultivation processes, and the relationships between the rate of growth, substrate consumption, substrate inhibition, product formation and product inhibition are crucial for optimizing and controlling photosynthetic or fermentation process (Bailey and Ollis, 1977).

Models to describe the carbon dioxide can be developed to improve the utilization of CO_2 in photobioreactors. These models are generally experimentally derived mathematical formulas that fit the cultivation data reasonably well. The kinetics can be linear or non-linear, single-phase or multiphase. Linear kinetic models include constant rate and first order kinetics. Non-linear kinetic models comprise exponential, logistic, second order and other defined function, e.g. Monod type kinetics. Curve-fitting algorithms can be used to solve these models, but a special care is necessary to determine the start point. A poor starting point can result in a divergent or a solution that no made physical sense (Motulsky and Ransnas, 1987).

Model validation is usually defined to mean “substantiation that a computerized model within its domain of applicability possesses a satisfactory range of accuracy consistent with the in-tended application of the model” (Schlesinger et al. 1979). A model should be developed for a specific purpose (or application) and its validity determined with respect to that purpose. Numerous sets of experimental conditions are usually required to define the domain of a model’s intended applicability. A model may be valid for one set of experimental conditions and invalid in another. A model is considered valid for a set of experimental conditions if the model’s accuracy is within its acceptable range, which is the amount of accuracy required for the model’s intended purpose. The amount of accuracy required should be specified prior to starting the development of the model or very early in the model

development process. Several versions of a model are usually developed prior to obtaining a satisfactory valid model (Henderson et al., 2007).

The ability to describe an object, material, process or action in mathematical terms is central to our paradigms for analysis and control. Detailed mathematical models are used by engineers in academia and industry to gain competitive advantage through applications of model-based process design, monitoring, control and optimization and represents an essential activity to photosynthetic or fermentative process development. Thus, building high quality and validated mechanistic models is a key activity in process engineering. When developing models that use a priori knowledge of physical, chemical or biological laws to propose (one or more) possible models, these laws dictate the model structure and invariably contain adjustable parameters (Neeleman, 2002).

In this regard, this study aimed to evaluate the use carbon dioxide consumption models in cyanobacteria cultivation for CO_2 biofixation process and biomass production. The focus is mathematical modeling of substrate consumption.

2 Materials and methods

2.1. Microorganism and culture conditions

Axenic cultures of *Aphanothece microscopica Nägeli* (RSMan92) were originally isolated from the Patos Lagoon estuary, from the state of Rio Grande do Sul, Brazil (32°01´S - 52°05´W). Stock cultures were propagated and maintained in synthetic BGN medium (Ripka et al., 1979) with the composition presented in Table 3.1 and pH 8.0. The incubation conditions used were 25ºC, photon flux density of 15μmol.m^{-2}.s^{-1}, and a photoperiod of 12h.

Table 3.1: Composition of synthetic medium BGN (pH 8.0)

Parameter	Value (mg/L)	Parameter	Value (mg/L)
$CaCl_2.6H_2O$	0.04	**Citric Ácid**	6.00
$CuSO_4.5H_2O$	0.079	**Na_2CO_3**	20.00
$ZnSO_4.7H_2O$	0.22	**K_2PO_4**	30.00
$Na_2MoO_4.2H_2O$	0.39	**$CaCl_2.2H_2O$**	36.00
Na_2EDTA	1.00	**NaCl**	72.00
$MnCl_2.4H_2O$	1.81	**$MgSO_4.7H_2O$**	75.00
H_3BO_3	2.86	**$NaNO_3$**	1500.00
Ammonium iron (III) citrate	6.00		

2.2. Photobioreactor design

The diagram of the experimental apparatus used is shown in Figure 3.1. The photobioreactor were constructed in 4mm thick glass with similar geometry, dimensions and working-volume (WV). The systems had an internal diameter of 7cm, height of 70cm and nominal volume of 2.4L. In the bubble column reactors (BCR) air was supplied through a 7cm diameter sinterized glass plate. The dispersion system for the reactor consisted of a 1.5cm diameter air diffuser located in the centre of the column. The reactor was continuously illuminated with sixteen 40W fluorescent daylight-type tubes (General Electric, Brazil), connected in parallel and located in a photoperiod chamber. Airflow into the photobioreactor was provided via a filtered air and pure CO_2 cylinder through Teflon tubing. The CO_2/air mixture was adjusted to achieve the desired concentration of carbon dioxide in the air-stream through three rotameters that measured the flow rates of the carbon dioxide, the air, and the mixture of gases, respectively.

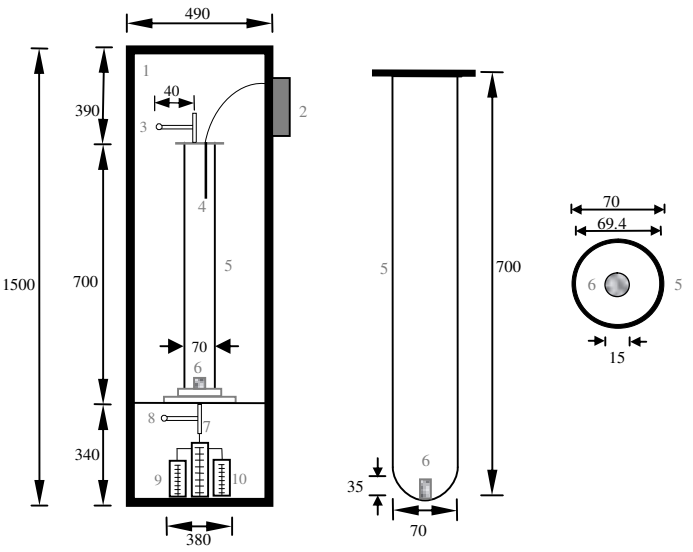

Figure 3.1: Photobioreactor diagram.

1: photoperiod chamber; 2: pH, temperature and CO_2 analyser; 3: gas exit sampler; 4: pH, temperature and CO_2 sensor; 5: photobioreactor; 6: gas diffuser;7: system controlling the flow rate and mixture of the gases; 8: Gas entrance sampler; 9: Air flow meter; 10: CO_2 flow meter. All dimensions in mm.

2.3. Obtaining the kinetic data

The experiments were carried out in bioreactors operating in a batch mode, fed with 2.4L of culture medium. The experimental conditions were as follows: initial cell concentration of 0.1g.L^{-1}, isothermal reactor operating at a temperature of 30ºC, photon flux density of 150µmol.m^{-2}.s^{-1}, and continuous aeration of 1VVM with the injection of air containing 15% carbon dioxide. The cell concentration and elemental composition of cells were monitored every 12h during the microbial growth phases. Residence times of up to 156h were considered for all the experiments. The tests were carried out in duplicate and the kinetic data referred to the mean of four repetitions.

2.4. Analytical methods

The cell concentration was evaluated gravimetrically by filtering a known volume of culture medium through a 0.45µm filter and drying at 60°C for 24 hours. The photon flux density was determined using a digital photometer (Spectronics, model XRP3000), measuring the light incident on the external reactor surface. The temperature was controlled using thermostats and measured using a polarographic probe (Mettler Toledo, lnPro5000 series). The flow rates of the carbon dioxide, air, and CO_2 enriched air were determined using rotameters (AFSG 100 Key Instruments). The composition of the elements of the *Aphanothece microscopica Nägeli* cells was determined using a Perkin Elmer 2400 CHNS/O element analyser. Two-milligram samples of biomass were oxidised at 1000ºC and the resulting gases were determined using a thermal conductivity probe for carbon. The standard used was acetanilide, with a composition of 71.09% carbon, 11.84% oxygen, 6.71% hydrogen, and 10.36% nitrogen.

Gas chromatography (GC) was used to determine CO_2 concentrations in airstreams. The equipment used was an Simple Chrom (Cromacon, Brasil) equipped with a column Porapack Q80/100, 6”x1/8“ S.S and a thermal conductivity detector. The operational conditions were as follows: injector and detector temperatures of 100 and 120ºC and column temperature of 70ºC. The carrier gas was hidrogenio with a flow rate of 30 mL/min. The sample volume injected was 100µL. The CO_2 removed was determined from samples taken from the gaseous phase of the system. The areas obtained using the integrator was compared with reference curves to determine the CO_2 concentrations.

3 Mathematical models and statistical validation

3.1. Cell Growth

The specific growth rates for different residence times are determined numerically by adjusting the cell concentration to a polynomial function, according to Equation 1 and represents a measure of how quickly a microbial population is growing (Ahamad & Holland, 1995):

$$\mu = \frac{1}{X}\frac{dX}{dt} \tag{1}$$

The exponential growth phase is determined from the graph of (ln X) *vs* (t) for different initial and final time limits, determining the start and duration of the exponential growth phase from the best correlation coefficients. The angular coefficient of the best correlation provides the value for μ_{max}, that is, the maximum specific growth rate.

The Modified Gompertz model (Zwietering et al., 1990) was used to determinate three biologically relevant parameters: lag time, specific growth rate, and the asymptotic value or maximum microalgae population. This model can be represented by:

$$y = C.exp^{-exp\left[\frac{\mu_{max}.exp}{C}(\lambda - t)+1\right]} \tag{2}$$

3.2. Substrate Consumption

The carbon dioxide consumption rate, q_{CO2}, is a measure of how quickly carbon dioxide was consumed. The carbon dioxide consumption rate for different residence times are determined numerically by this equation (Ohtaguchi & Wijanarko, 2002).

$$q_{CO2} = Q_g \frac{y_{CO2,e} - y_{CO2,s}}{V.R.T}\frac{M_{CO2}.P_T}{X} \tag{3}$$

Such models are available in literature to represent the growth rate as a function of limit substrate or inhibit substrate. In this regard, six models have been tested to predict the maximum substrate consumption rate (q_{CO2_max}), Saturation constant (K_x) and Inhibition constant (K_I). Webb presented his model in 1964, Eq. 4, with an introduction of an auxiliary inhibition constant. Aiba et al,

(1968), Eq. 5, demonstrated a far simpler model in terms of number of parameters, but presenting an exponential. Andrews, 1968, Eq. 6, presented his model as simple in mathematical terms. In 1969, Yano e Koga , Eq. 7, formulated their model with a quadratic auxiliary inhibition constant and in 2004. Ierusalimsky and Chen models, Eq. 8 and 9, were expressed in Ohtaguchi & Wijanarko, 2004. The follow equations show these models with modifications (by Ohtaguchi & Wijanarko, 2004) to predict the parameters as a function of continuous feed.

$$q_{CO2} = q_{CO2\_max} \frac{X\left(k_X + \frac{X}{k_{I,2}}\right)}{k_X + X\left(k_X + \frac{X}{k_I}\right)} \tag{4}$$

$$q_{CO2} = q_{CO2\_max} \frac{Xe^{-X/k_I}}{X + k_X} \tag{5}$$

$$q_{CO2} = q_{CO2\_max} \frac{X}{X\left(k_X + \frac{X}{k_I}\right) + k_X} \tag{6}$$

$$q_{CO2} = q_{CO2\_max} \frac{X}{k_X + X\left(k_X + \frac{X}{k_I} + \frac{X^2}{k_{I,2}^2}\right)} \tag{7}$$

$$q_{CO2} = q_{CO2\_max} \frac{X}{k_X + X} \frac{1}{k + \frac{X}{k_I}} \tag{8}$$

$$q_{CO2} = q_{CO2\_max} \frac{X\left(k + \frac{X}{k_I}\right)}{X + k_X + \left(k_{X2} X\right)^2} \tag{9}$$

3.3. Mathematical Resolution and Statistical Validation

Least Square was used in Microsoft Excel® XP Software to solve the models through an iterative procedure (solver - Newton Method) which determines the minimization of the least value of the square error sums between the empirical and predicted values. Because a poor starting point can result in a divergence or wrong solution, a reasonable initial estimate for the coefficient was obtained by the graph (ln X) vs. (t). Indices of performance of the predictive models were calculated by the expressions in Table 3.2 (Ross, 1996):

Table 3.2: Parameters for statistical validation.

Parameter	Significance	Equation	Expected Value
RMSE	Measure of the concordance between the experimental data and the model	$RMSE = \sqrt{\frac{\sum (obs - pred)^2}{n}}$	0
Bias Factor ***	objective indication of better performance of the model	$B_f = 10^{\frac{\sum log(pred/obs)}{n}}$	1 >1 = over estimation <1= under estimation
Accuracy Factor	difference between the observed mean average values and the predicted values	$A_f = 10^{\frac{\sum \lvert log(pred/obs) \rvert}{n}}$	1 >1 = over estimation <1= under estimation
%SEP	Quality Assessment prediction	$\%SEP = \frac{100}{mean\ obs}\sqrt{\frac{\sum (obs - pred)^2}{n}}$	0

*** Models describing growth rates with a Bf in the range of 0.9–1.05 could be considered good, in the range of 0.7–0.9 or 1.06–1.15 considered acceptable, and <0.7 or >1.15 considered unacceptable.

The transfer carbon dioxide rate to biomass was calculated by the following equation (Ohtaguchi & Wijanarko, 2004)

$$TRC = q_{CO2}.X \qquad (14)$$

4 Results and Discussions

A typical time course for batch cultivation is determined with respect to cell mass. The process can be divided into four principal phases: the initial lag phase occurs due to regulatory phenomena of the microorganisms as the inoculum adapts to the conditions in the bioreactor and there is excessive substrate concentration in this period; the next phase is called exponential growth, where the specific growth rate is constant and maximum; then there is the stationary phase with an specific growth rate constant and null, brought about by substrate limitation, product inhibition, or other phenomena; followed by a declining phase in which the cell mass decreases due to lysis or endogenous metabolism (Schimidell et al., 2005). The models must predict these phases reasonably well in order to achieve good fitting of data. Mathematical modeling has been widely used to predict cellular growth by estimating the maximum specific growth rate, maximum cell concentrations, and duration of the lag phase, which are all necessary in the study of microbial growth and for use in industrial microbiology. The values obtained by the Gompertz modified model were $\mu_{max}=0.72d^{-1}$, $\lambda=9h$, $X_{max}=5.1g.L^{-1}$ (RMSE=0.067, %SEP=2.642, $B_f=1.008$ e $A_f=1.056$) and Figure 3.2 provides the fit of the model to the experimental data.

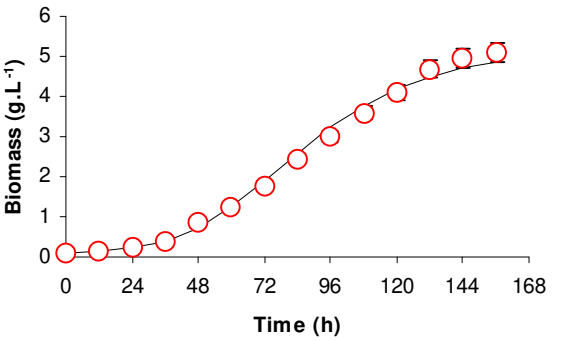

Figure 3.2: Fit of the experimental data to Modified Gompertz model.

It must be taken into account that the biological fixation of CO_2 is an extremely complex process and is not only in the form of biomass. Other products of photosynthetic metabolism are involved in these processes, such the formation of exocellular proteins and carbohydrates associated with the catalysis of chemical species in the precipitated carbonate and bicarbonate formation and volatile organic compound (VOCs) formation. Many compounds can be formed in photosynthetic

reactions, associated with high CO_2 conversion rates (Jacob-Lopes, 2007a). In this regard, the biological fixation of CO_2 is very interesting in relation to reducing climate changes.

The results shown in Figure 3.3 reveal a loss of 83.3% CO_2 in the reactor feed (average). Therefore it is necessary to study in detail the amount of gas in the inlet and the efficiency obtained using one or more reactors. The profile of CO_2 loss can be correlated with cell growth because this value decreases with time until 110h of experiment (end of exponential growth phase). After this period, the loss of CO_2 begins increase due to the decrease in cell growth and number of viable cells inside of the reactor.

In this regard, the use of mathematical models to predict the best conditions for CO_2 biofixaton becomes necessary. It can be said that the number of reactors to be arranged in series should be determined by the cost-benefit achievable through this combination. It should be necessary to know that this loss is a function of the microorganism, the CO_2 concentration at the inlet and the configuration and operation of the photobioreactor (Eriksen et al., 2007; Fan et al., 2008). Cheng et al., (2006) obtained 0.08g.m^{-3}.h^{-1} in 1% CO_2 in a membrane photobioreactor with *Chlorella vulgaris*, reporting a removal efficiency peak of 55.3%, for up to 0.15% CO_2. Chiu et al., (2008) using a bubble column reactor, obtained a maximum disposal capacity of 0.72 gm^{-3}.h^{-1} in 15% CO_2. However, in this study, the maximum removal capacity was verified as almost 78.0 gCO_2.m^{-3}.h^{-1} as an overall average. This value is higher than those reported in other studies.

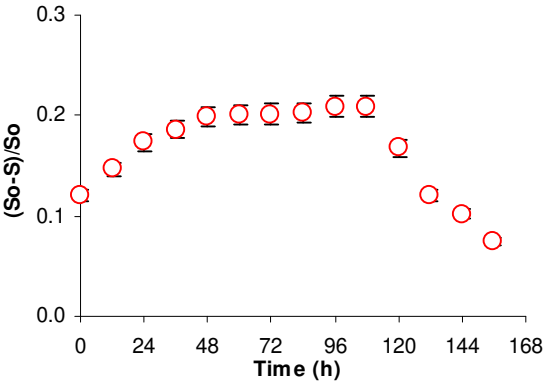

Figure 3.3: Loss of CO_2 feed in the reactor.

Richardson e Jackson (2007) reported that cells of cyanobacteria growing exponentially in turbulent systems tend to form clusters in the collision cell. The rate of formation of these clusters depends primarily of the abundance and size of cells. According to these authors, these characteristics determine the pattern of absorption and desorption of inorganic carbon in aqueous systems. The formation of cell clusters provides a low capacity to carbon dioxide removal, due to problems of agitation and mixing in the reactor reduced the combined area per unit volume of cell which is capable of capturing light energy for the subsequent reaction of carbon atom fixation.

Based on the results of CO_2 loss by the system, an evaluation of growth models based on substrate consumption is required to define optimum conditions process for industrial use, aiming to maximize CO_2 removal. The models tested here are the result of some considerations that should be mentioned, such as the assumption that only one substrate is limiting, or the others were fed in excess, these are also models of unstructured and unsegregated model. Figure 3.5 provides the fit of the models to experimental data. Table 3.3 presents the performance indicators of the models tested.

Statistical analysis of the models shows that the Webb and Tan models cannot describe substrate consumption data. Thus, it was necessary to evaluate the characteristics of each model and the significance of the physics results in order to select the most appropriate prediction model. The selection criteria can be complexity, use and accuracy of the model and potential for physiological significance, interpretation and number of model parameters (Phua and Davey, 2007) and, in this case, the criteria used was ease of synthesis and use. The Andrews model describes carbon dioxide consumption data both in terms of statistical accuracy and ease of use when compared to other functions (McDonald and Sun, 1999). Therefore, the Andrews model was selected to predict the carbon dioxide substrate consumption and was considered statistically sufficient and robust enough to describe the data of the tested organism.

As described in the literature (Bailey & Ollis, 1977; Blanch and Clarck, 1997), there are many models to describe cell growth or substrate consumption and each culture has a better adherence to a particular model. It is not expected that all the models will achieve a high level of proximity between the actual and predicted values, thus the existence of better performance between the models tested and the fact that some of them can not be used for prediction represents confirmation of the need to have tested many models in order to describe the behavior of the microorganism.

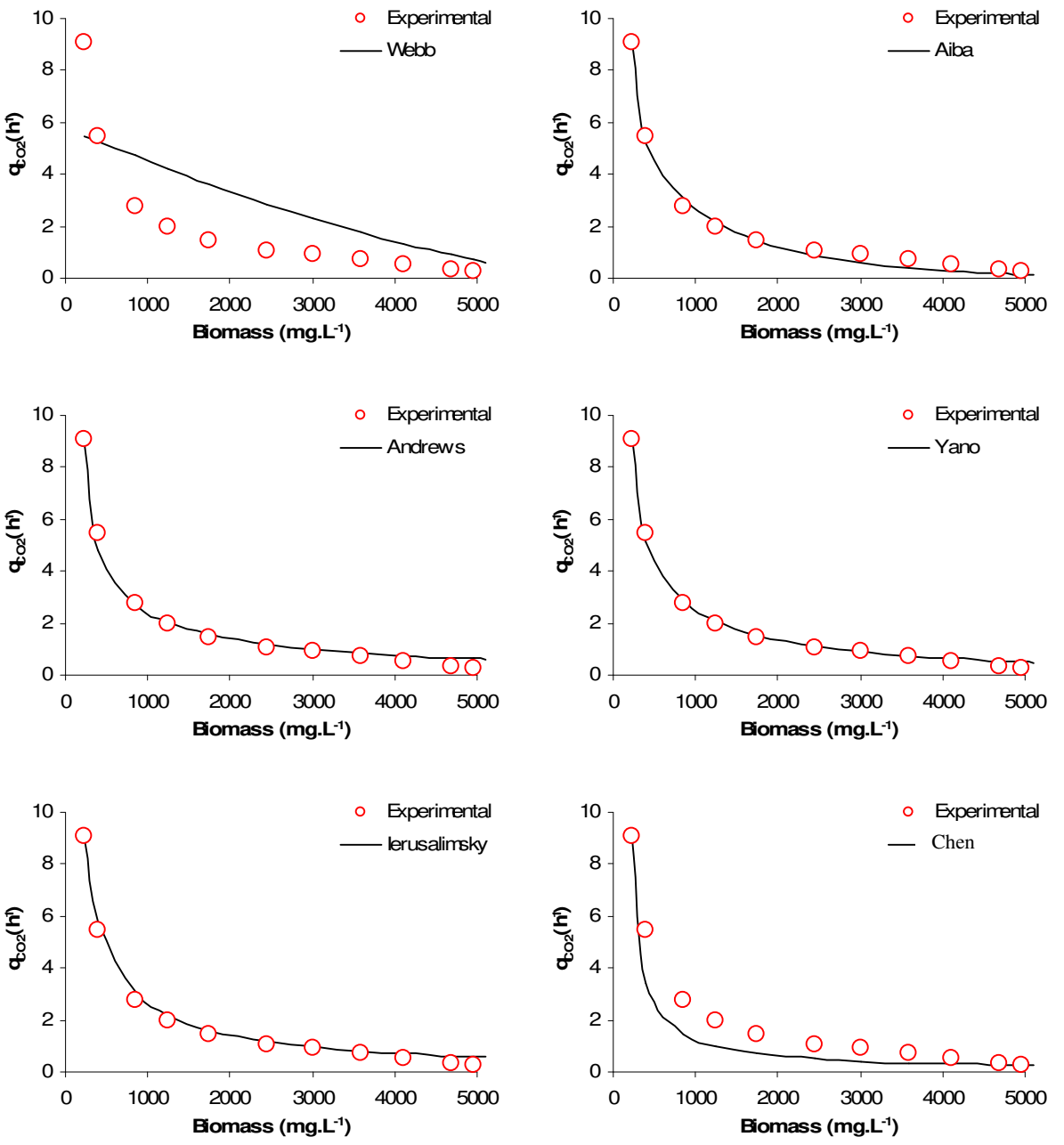

Figure 3.4: Fit of the experimental data to substrate consumption models.

Table 3.3: Statistic Validation for the models.

Parameters	Webb	Aiba	Andrews	Yano	Ierusalimsky	Chen
RMSE	1.56	0.16	0.21	0.11	0.15	0.78
%SEP	53.06	5.45	10.03	3.60	5.00	26.59
Bf	1.32	0.97	1.01	1.01	1.04	0.73
Af	1.44	1.07	1.04	1.02	1.04	1.37

The value of the saturation constant, Kx, and the inhibition constant, Ki, have a similar meaning to the Ks and Ki of the Michaelis-Menten model, thus the value in $mg.L^{-1}$ of biomass in the rate of consumption of CO_2 reaches half of its maximum value and value in $mg.L^{-1}$ of biomass which starts the process of inhibition, respectively. The results obtained with the Andrews model were: reactor $q_{CO2\_max} = 6.86\ h^{-1}$, $Kx = 149mg.L^{-1}$, $Ki = 498mg.L^{-1}$. Therefore, the Modified Gompertz model was used to determine the length of residence in which Ki and Kx are met. Kx to the concentration of $298mg.L^{-1}$ was obtained in 30h. For Ki, the concentration of $498mg.L^{-1}$ was obtained in 42h. The formation of cell clusters in the cultivation of microorganisms was observed, as from 500 $mg.L^{-1}$ in several experiments conducted under similar conditions of CO_2 supply, temperature and light intensity (Jacob-Lopes, 2007), and above, the formation of these clusters promotes a reduced ability to remove carbon dioxide, due to problems of agitation and mixing in the reactor diminishing the combined area per unit volume of cell capable of capturing light energy for the subsequent reaction of carbon atom fixation. It is should be mentioned that the formation rate of these clusters depends primarily on the abundance and size of cells, thus maintaining the culture at an early stage of development and low cellular concentration facilitates the flow of absorption and desorption of inorganic carbon in aqueous systems.

The data presented above define the range of best performance of the microorganism in the process of sequestration and biological fixation of CO_2, but it is necessary to clarify that although the region of better performance of cyanobacteria is between 300 and 500 $mg.L^{-1}$, the range of best performance of the system is between 1000 and $3000mg.L^{-1}$. This is due to the compensatory effect between the increase of biomass and decrease in performance of the microorganism. These values were calculated according to equation 14. A better visualization of the compensatory effect of biomass is obtained when comparing the value obtained by the results of the model (q_{CO2_max} and 2.Kx) with the maximum value obtained in terms of biomass in this reactor.

Figure 3.6 shows these comparisons and maps TRC in terms of biomass. The relevance of these data is the need to keep this process in a state for industrial application, thus requiring knowledge of the best period to start the change from batch to continuous as regards the power of synthetic medium and the removal of biomass. One factor that should be taken into account is the fact that the biomass produced in its composition bears characteristics of commercial interest, i.e., a significant size of proteins, lipids, carbohydrates, nucleic acids and pigments, and can therefore be used as an ingredient in food for the feeding and extraction of biomolecules in the production of biofuels (Jacob-Lopes et al., 2007b, Zepka et al., 2008, Gressel, 2008). Thus, linking the performance of the process and the formation of biomass, the best period to start the dilution of the medium (0.0512 h^{-1}) is 96 hours of time of residence in both reactors. It can be commented that in this case the production of biomass by the system will be approximately 3.00g.L^{-1}.h^{-1} against 0.35 g.L^{-1}.h^{-1} in the case of dilution beginning at a residence time of 30h

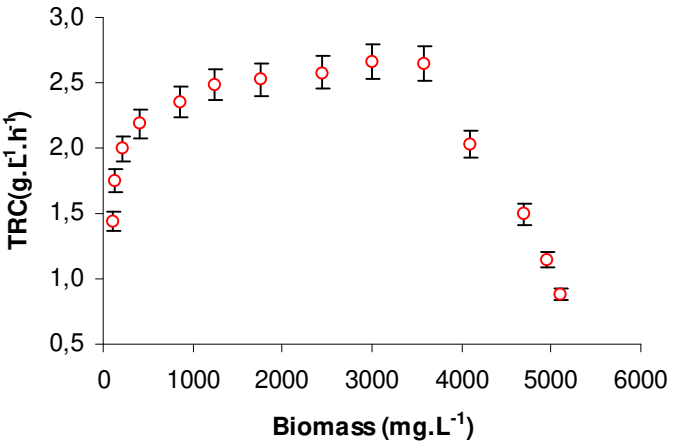

Figure 3.5: Transfer of Carbon dioxide rate.

5 Conclusion

The results reveal a loss of 12.5% of CO_2 in the reactor feed (average). Therefore, it is necessary to study in detail the amount of gas at the inlet and the efficiency obtained with the dispersor system or using one or more reactors. Hence, the need to use mathematical models to predict the best

conditions for CO_2 biofixaton. As a result of statistical analysis, the Andrews model was selected to be considered statistically sufficient and robust. The results obtained with this model were q_{CO2_max} = 6.86 h^{-1}, Kx = 149mg.L^{-1}, Ki = 498mg.L^{-1}. Therefore, an advantage was observed in defining the beginning of the dilution system in 96 hours of time of residence for higher system performance combined with the increased formation of biomass, although this is not the best performance condition of the microorganism. Given that the biomass produced is of the order 3.00$g_{biomassa}$.L^{-1}.h^{-1} and the maximum TRC of 2.67 $g_{CO2}L^{-1}.h^{-1}$ and the potential use of this system in processes of biological dioxide carbon fixation was demonstrated.

6 Notation

Acronyms

Mean obs	Mean of observed values
Obs	Observed values
Pred	Predicted values by models

Notations

A	ln (X_{max}/X_0)	-
C_C	Fraction of carbon in biomass	-
C_i, C_o	Inlet and outlet substrate concentration	$mg.L^{-1}$
K_I, K_{I2}	Inhibition Constant and Auxiliary Inhibition Constant	$mg.L^{-1}$
K_x	Saturation Constant (relation of biomass)	$mg.L^{-1}$
n	Number of tests	-
M_{CO2}, M_C	Molecular weight of CO_2, Molecular weight of Carbon	g
P	Ambient Pressure	atm
Q_g	Volumetric gas flow rate measured at gas inlet	$L.h^{-1}$
q_{CO2}, q_{CO2_max}	Incident CO_2-fixation rate; Maximum CO_2-fixation	h^{-1}
R	Universal gas Constant (0,082)	$atm.L.(mol.K)^{-1}$
T	Culture medium temperature	K
t	Residence time	h
V	Volume of culture medium	L
X_0, X_{max}, X	Initial Cell concentration, max concentration and in the time t	$mg.L^{-1}$
y	ln (X_t/X_0)	-
$y_{CO2,e}$, $y_{CO2,s}$	CO_2 concentration of input gas and of output gas	fraction

Greek Symbols

λ	Lag phase duration	h^{-1}
μ, μ_{max}	Specific and Maximum specifi growth rate	h^{-1}

7 References

AHMAD, M.N., HOLLAND, C.R. Growth kinetics of single-cell protein in batch fermenters. Journal of Food Engineering, 26, 443–452, 1995.

AIBA, S., HUMPHREY, A.E, MILLIS, N.F. Biochemical engineering. Academic press, 1st edition, 1968.

ANDREWS, J.F. A mathematical model for the continuous culture of microorganisms utilizing inhibitory substance. Biotechnology. Bioengineering, 10, 707-723, 1968.

BAILEY, J.E., OLLIS, D.F. Biochemical engineering fundamentals. New York, N.Y, McGraw-Hill Inc., 1977.

BLANCH, H.W., CLARK, D.S. Biochemical engineering. Marcel Dekker, Inc., New York, 1997.

CHENG, L., ZHANG, L., CHEN, H., GAO, C. Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor. Separation and Purification Technology, 50, 324–329, 2006.

CHIU, S.Y., KAO, C.Y., CHEN, C.H., KUAN, T.C., ONG, S.C., LIN, C.S. Reduction of CO2 by a high-density culture of Chlorella sp. in a semicontinuous photobioreactor. Bioresource Technology, 99, 9, 3389-3396, 2008.

DERNER, R.B., OHSE, S., VILLELA, M., CARVALHO, S.M., FETT, R.. Microalgas, produtos e aplicações. Ciência Rural, Santa Maria, 36, n.6, p.1959-1967, 2006.

ERIKSEN, N.T., RIISGARD, F.K., GUNTER, W.G., IVERSEN, J.J.L. On-line estimation of O2 production, CO2 uptake, and growth kinetics of microalgal cultures in a gas-tight photobioreactor. J. Appl. Phycol., 19, 161-174, 2007.

FAN, L.H., ZHANG, Y.T., ZHANG, L., CHEN, H.L. Evaluation of a membrane-sparged helical tubular photobioreactor for carbon dioxide biofixation by Chlorella vulgaris Journal of Membrane Science. In Press. doi:10.1016/j.memsci.2008.07.044, 2008.

GRESSEL, J. Transgenics are imperative for biofuel crops. Plant Science, 174, 246-263,2008.

HSUEH, H.T., LI, W.J., CHEN, H.H., CHU, H. Carbon bio-fixation by photosynthesis of *Thermosynechococcus sp.* CL-1 and *Nannochloropsis oculta*. Journal of Photochemistry and Photobiology B: Biology, 95, p.33–39, 2009.

JACOB-LOPES, E., LACERDA, L.M.C.F., FRANCO, T.T. Biomass production and carbon dioxide fixation by Aphanothece microscopica Nägeli in a bubble column photobioreactor, Biochemical Engineering Journal, 40, 27-34, 2008.

JACOB-LOPES, E., SCOPARO, C.H.G., FRANCO, T.T. Rates of CO2 removal by Aphanothece microscopica N¨ageli in tubular photobioreactors. Chemical Engineering and Processing, 47, 1365–1373, 2008.

KAJIWARA, S., YAMADA, H., NARUMASA, O. Designof the bioreactor for carbon dioxide fixation by Synechococcus PCC7942. Energy Convers. Mgmt, 38, 529-532, 1997.

MCDONALD, K., SUN, D.W. Predictive food microbiology for the meat industry. International Journal of Food Microbiology, 52; 1-27, 1999.

MCMEEKIN, T.A., OLLEY, J.N., ROSS, T., RATKOWSKY, D.A.; Predictive Microbiology: Theory and Application; Wiley, New York; (1993).

Molina et al., 2001).

MOLINA GRIMA, E.; FERNANDÉZ, F.G.A.; CAMACHO, F.G.; CHISTI, Y. Photobioreactors: light regime, mass transfer, and scale up. Journal of Biotechnology, n.70, p.231-247, 1999.

MOTULSKY, H.J., CHRISTOPOULOS, A. Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting. GraphPad Software Inc., San Diego CA, www.graphpad.com, 2003.

MOTULSKY, H.J., RANSNAS, L.A., 1987. Fitting curves to data using nonlinear-regression – a practical and nonmathematical review; Faseb Journal; 1; 365–374.

NAKASHIMA, S.M.K., ANDRÉ, C.D.S., FRANCO, B.D.G.M., Review: Basici Aspects of Predictive Microbiology, Brazilian Journal of Food Technology, 3, 41-51, 2000.

NEELEMAN, R. Biomass performance: monitoring and control in bio-pharmaceutical production. Thesis Wageningen University, 2002.

NETER, J., WASSERMAN, W., KUTNER, M.H., Applied linear statistical models: regression, analysis of variance, experimental designs. 3.ed, Homewood: Richard d. Irwing, 1127, 1996.

PHUA, S.T.G., DAVEY, K.R. Predictive modeling of high pressure (<700MPa)–cold pasteurization (<25°C) of Escherichia coli, Yersinia enterocolitica and Listeria monocytogenes in three liquid foods. Chemical Engineering and Processing, 46, 458–464, 2007.

REVAH, S., MORGAN-SAGASTUME, J.M. Methods for odor and VOC control. *Biotechnology for Odour and Air Pollution*, Springer-Verlag, Heidelberg, Germany pp. 29–64, 2006.

RICHARDSON, T.L., JACKSON, G.A. Small phytoplankton and carbon export from the surface ocean. Science, 315, 838-840, 2007.

RIPPKA, R., DERUELLES, J., WATERBURY, J.B., HERDMAN, M., STANIER, R.Y. Generic Assignments Strain Histories and Properties of Pure Cultures of Cyanobacteria. Journal of General Microbiology, 111, 1-61, 1979.

ROSS, T., Indices for performance evaluation of predictive models in food microbiology. J. Appl. Bacteriol., 81, 501–508, 1996.

SCHIMIDELL, W., LIMA, U.A., AQUARONE, E., BORZANI, W. Biotecnologia Industrial Volume 2, Editora Edgar Blüncher, 2005.

SCHOPF, J.W. Ritmo e modo da evolução microbiana pré-cambriana. Estud. Av., 9, 23, p.195-216, 1995.

SCHWARZ, R., FORCHHAMMER, K. Acclimation of unicellular cyanobacteria to macronutrient deficiency: emergence of a complex network of cellular responses. Microbiology, 151, 2503–2514, 2005.

WANG, B., LI. Y., WU, N., LAN, C.Q. CO2 bio-mitigation using microalgae. Appl Microbiol Biotechnol,79, 707–718, 2008.

WEBB, F.C. Biochemical Engineering, Van Nostrand, 1964.

WIJANARKO, A., OHTAGUCHI, K. Carbon dioxide removal and biomass production by Anabaena cylindrica IAM M1 using reactor in series. Studies in Surface Science and Catalysis, 153, 461-468, 2004.

WIJANARKO, A., OHTAGUCHI, K. Elevation of the efficiency of cyanobacterial Carbon Dioxide removal by Monoethanolamine solution. Technology, 8, 267-285, 2002.

WOODWARD, F.L., BARDGETT, R.D., RAVEN, J.A., HETHERINGTON, A.M. Biological approaches to global environment review-change mitigation and remediation. Current Biology, 19, r615–R623,2009

YANG, H., XU, Z., FAN, M., GUPTA, R., SLIMANE, R.B., BLAND, A., WRIGHTS, I.. Progress in carbon dioxide separation and capture: A review. Journal of Environmental Sciences, 20, p.14–27, 2008.

YANO, T., KOGA, S. Dynamic behaviour of the chemostat subject to substrate inhibition. Biotechol. Bioeng., 11, 2, 139-153, 1969.

YU, S., CLARK, O.G., LEONARD, J.J. A statistical method for the analysis of nonlinear temperature time series from compost. Bioresource Technology, 99, 1886–1895, 2008.

ZEPKA, L.Q., JACOB-LOPES, E., GOLDBECK, R. QUEIROZ, M.I. Production and biochemical profile of the microalgae Aphanothece microscopica Nägeli submitted to different drying conditions. Chemical Engineering and Processing, 47, 1305-1310, 2008.

ZWIETERING, M.H., JONGENBURGER, I., ROMBOUTS, F.M., VAN'T RIET, K., Modeling of the bacterial growth curve. Appl. Environ. Microbiol., 56, 1875–1881, 1990.

Capítulo 5

Conclusão Geral

Conclusão

A presença de compostos inibidores de crescimento celular associado com a limitação de nutrientes essenciais para o aumento celular de cianobactérias foi evidente no efluente líquido testado. A suplementação do efluente líquido com 25% (w/v) dos sais de BGN é uma boa estratégia para a viabilização do uso desse efluente como base de cultivo celular e fixação biológica de CO_2. Nessa condição operacional, a melhor taxa de crescimento específico foi de $1{,}12 d^{-1}$. O modelo Gompertz Modificado foi indicado para descrever o comportamento do microorganismo testado, como evidenciado pelos critérios estatísticos. As predições de máxima produtividade indicam a possibilidade de se obter $1{,}41 kg_{biomassa}/L_{reator}$ em 1000h de operação com uma fixação de $2{,}61 kg_{CO2}/L_{reator}$.

O consumo de dióxido de carbono foi avaliado em experimentos realizados apenas com meio BGN e tiveram como finalidade avaliar parâmetros como taxa máxima de consumo de CO_2, constante de saturação da biomassa e constante de inibição, sendo aplicados modelos de consumo de substrato (Webb, Aiva, Yano & Koga, Andrews, Chen e Ierusalinsky) para analisar os resultados e definir a condição de melhor concentração de biomassa para promover uma elevada fixação biológica de CO_2. Foi observado uma perda de aproximadamente 12,5% dos 15% de CO_2 alimentado no reator (média), confirmando a necessidade de se estudar modelos matemáticos para prever as melhores condições de alimentação dos gases no reator para que seja obtida a maior eficiência do sistema. Em função da análise estatística dos resultados obtidos com os modelos pode-se dizer que o modelo Andrews foi selecionado por ser considerado estatisticamente suficiente e robusto para predizer o consumo de dióxido de carbono pela *Aphanothece microscopica Nägeli* cultivada em fotobiorreatores. Os resultados obtidos com este modelo foram $q_{CO2\_max} = 6.86\ h^{-1}$, $Kx = 149 mg.L^{-1}$ (30h de cultivo), $Ki = 498 mg.L^{-1}$ (42h de cultivo). Avaliando-se esses dados e a taxa de remoção de carbono que é diretamente influenciada pela biomassa, observou-se a vantagem em se definir o início da diluição do sistema em 96h de tempo de residência para obter maior desempenho do sistema aliado à maior formação de biomassa, embora essa não seja a melhor condição de desempenho do microorganismo. Em face disso a biomassa produzida será da ordem de $3{,}00 g_{biomassa}.L^{-1}.h^{-1}$ e máxima TRC na forma de biomassa de $2{,}67 g_{CO2}.L^{-1}.h^{-1}$mostrando a potencialidade de uso desse sistema em processos de fixação biológica de dióxido de carbono.

Capítulo 6

Sugestões para trabalhos futuros

Sugestões para Trabalhos Futuros

- Melhorar a transferência de massa.
    - Estudar diferentes configurações de fotobiorreatores;
    - Estudar diferentes sistemas de dispersão de gases;
    - Estudar diferentes vazões de entrada dos gases.
- Avaliar o cultivo em água do mar.
    - Verificar a adaptação em diferentes concentrações salinas e temperaturas.
- Determinar o balanço de massa do carbono no sistema.
- Determinar formas de separação da biomassa.
- Determinar diferentes usos para a biomassa.
- Efetuar o aumento de escala
    - Estudar as condições que minimizem os processos de foto-inibição, foto-oxidação e foto-limitação em cultivos outdoor;
    - Verificar o melhor regime de operação (batelada, batelada alimentada e contínuo);
    - Detalhar as operações unitárias para um processo de produção de biomassa;
    - Avaliar a viabilidade econômica do processo.

Capítulo 7

Anexos

ANEXO I

Biomass production and carbon dioxide fixation by Aphanothece microscopica Nägeli in a bubble column photobioreactor

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Biomass production and carbon dioxide fixation by *Aphanothece microscopica Nägeli* in a bubble column photobioreactor

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Abstract

The objective of the present study was to evaluate the growth kinetics of *Aphanothece microscopica Nägeli* under different conditions of temperature, light intensity and CO_2 concentration. The growth kinetics of the microorganism and carbon biofixation were evaluated using a central composite design, considering five different temperature levels (21.5, 25, 30, 35 and 38.5 °C), light intensities (0.96, 3, 6, 9 and 11 klux) and carbon dioxide concentrations (3, 15, 25, 50 and 62%). The results obtained showed the effects of temperature, light intensity and CO_2 concentration ($p < 0.05$) on the photosynthetic metabolism of the microorganism. Response surface methodology was adequate for process optimisation, providing a carbon fixation rate to the order of 109.2 mg L^{-1} h^{-1} under conditions of 11 klux, 35 °C and 15% carbon dioxide, representing an increase of 58.1% as compared to the conditions tested initially.

Keywords: Carbon dioxide sequestration; Photobioreactor; Cyanobacteria

1. Introduction

Innumerable research projects have suggested that the biofixation of CO_2 by cyanobacteria in photobioreactors is a sustainable strategy, since carbon dioxide can be incorporated into the molecular structure of cells in the form of proteins, carbohydrates and lipids by way of photosynthetic reactions. The advantages of these processes are related to the greater photosynthetic efficiency of these systems when compared to higher plants, the resistance of these microorganisms to high carbon dioxide concentrations and the possibility of controlling the culture growth conditions. In addition, the biomass produced by the bioconversion of carbon dioxide allows one to obtain products of high added value, such as, fatty acids, biodiesel, biogas and organic fertilizers, as a function of the microalgal species used and the effluent to be treated [1–3].

According to Lee et al. [4] and Jacob-Lopes et al. [5], only a small fraction of the carbon dioxide injected into the photobioreactors is incorporated into the microalgal biomass. According to these authors, other products of photosynthetic metabolism, such as the formation of extracellular biopolymers, precipitates of chemical species such as carbonates and bicarbonates and volatile organic compounds (VOCs), are more highly represented in the carbon dioxide transformation processes of these systems, substantially increasing the fixation rates.

Aphanothece microscopica Nägeli is a cyanobacterium characteristic of the estuaries in southern Brazil, belonging to the family *choroococaceae* and forming blue-green colonies adapted for floating. It shows a macroscopic, amorphous structure with abundant, firm and rigid mucilage, and non-cylindrical ellipitical adult cells measuring 9.0–9.5 μm × 4.0–4.2 μm, approximately 2.2 times longer than they are wider [6]. Previous studies have shown the potential of applying this microorganism in bioremediation and for the production of single-cell protein (SCP) [7–9].

Thus the objective of the present study was to evaluate distinct operational conditions for photobioreactors, expressed in terms of the temperature, light intensity and carbon dioxide concentration, aimed at maximizing carbon fixation in the cells of the cyanobacterium *Aphanothece microscopica Nägeli*.

2. Materials and methods

2.1. Microorganism and culture medium

Unialgal cultures of *Aphanothece microscopica Nägeli* (RSMan92) were originally isolated from the Patos Lagoon

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Nomenclature

C_c	percent carbon in the biomass (%)
(L)	linear effect
M_{CO2}	molecular weight of CO_2
M_C	molecular weight of carbon
(Q)	quadratic effect
R^2	coefficient of determination
R_C	carbon fixation rate (mg L^{-1} h^{-1})
t	residence time in $t=n$ (h)
t_0	residence time in $t=0$ (h)
t_g	generation time (h)
t_{log}	duration of logarithmic growth phase (h)
X_m	maximum cell concentration (mg L^{-1})
X_0	initial cell concentration (mg L^{-1})
X_1, X_2, X_3	independent variables of the statistical model
Y	variable response

Greek letters

β	parameters of the statistical model
μ_{max}	maximum specific growth rate (h^{-1})

estuary, Rio Grande do Sul State, Brazil (32°01′S–52°05′W). Stock cultures were propagated and maintained on synthetic BGN medium [10] with the following composition: $K_2HPO_4{\cdot}3H_2O$ (0.040 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.075 g L^{-1}), EDTA (0.001 g L^{-1}), H_3BO_3 (2.860 g L^{-1}), $MnCl_2{\cdot}4H_2O$ (1.810 g L^{-1}), $ZnSO_4{\cdot}7H_2O$ (0.222 g L^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (0.390 g L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.079 g L^{-1}), $CaCl_2{\cdot}6H_2O$ (0.040 g L^{-1}), $NaNO_3$ (150 g L^{-1}) $C_6H_8O_7{\cdot}H_2O$ (0.006 g L^{-1}), ammonium and iron citrate (0.006 g L^{-1}), pH 8.0. The conditions used were 25 °C, 1 klux of light intensity and a photoperiod of 12 h.

2.2. Photobioreactor

The diagram of the experimental apparatus used in this study is shown in Fig. 1. Measurements were made in a bubble column photobioreactor. The system was built in 4 mm thick glass, an internal diameter of 7.5 cm, height of 75 cm and nominal working volume of 3.0 L. The dispersion system for the reactor consisted of a 1.5 cm diameter air diffuser located in the centre of the column. The reactor was continuously illuminated with sixteen 20 W fluorescent lamps connected in parallel, located in a photoperiod chamber. Different numbers of lamps on each side of the photoperiod chamber were combined to give the desired light intensity. Airflow into the photobioreactor was provided via filtered air and pure CO_2 cylinder through Teflon tubing. The CO_2/air mixture was adjusted to achieve the desired concentration of carbon dioxide in the airstream, through three rotameters that measured the flow rates of the carbon dioxide, the air and the mixture of gases, respectively.

2.3. Obtaining and analysis of the kinetic data in an experimental photobioreactor

The experiments were carried out in bioreactors operating with an intermittent regime, fed on 3.0 L synthetic BGN medium. The test conditions were: initial cell concentration of 100 mg L^{-1}, isothermal reactor operating under different temperatures and light intensities and continuous aeration of

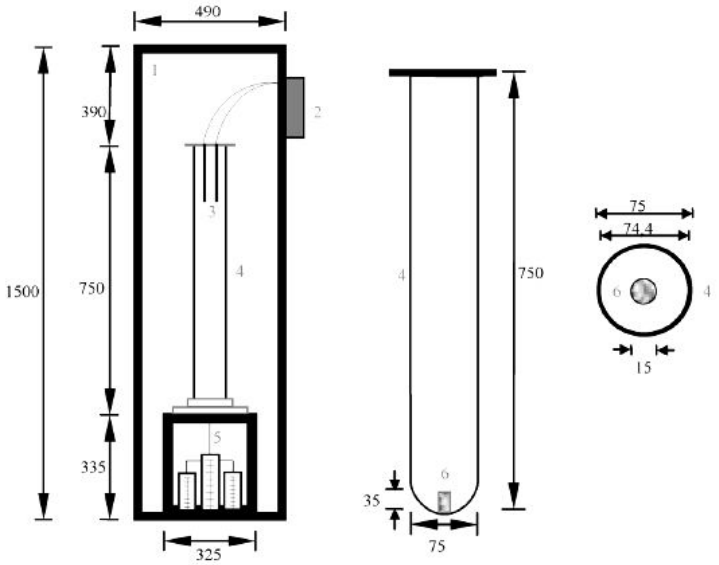

Fig. 1. Photobioreactor diagram. 1: Photoperiod chamber; 2: pH, temperature and CO_2 analyser; 3: pH, temperature and CO_2 sensors; 4: photobioreactor; 5: system controlling the flow rate and mixture of the gases; 6: gas diffuser. All dimensions in mm.

Table 1
Values of the independent variables for the different levels of the design

Independent variable	Symbol	Level				
		−1.68	−1	0	1	+1.68
Temperature (°C)	X_1	21.5	25	30	35	38.5
Light intensity (klux)	X_2	0.96	3	6	9	11
CO_2 concentration (%)	X_3	3	15	25	50	62

3 L min^{-1} of air supplemented with CO_2. The cell concentration was monitored every 12 h during the microbial growth phases. Residence times of up to 156 h were considered for all the experiments.

Response surface methodology was used to determine the optimal conditions for carbon fixation as a function of three experimental factors (temperature, light intensity and concentration of carbon dioxide enriched air). This methodology is widely used for controlling the effects of parameters in many processes. Its usage decreases the number of experiments, reducing time and material resources. Furthermore, the analysis performed on the results is easily carried out and experimental errors are minimized. The statistical method measures the effects of changes in operating variables and their mutual interactions on the process by way of an experimental design. The three steps used in the experimental design included statistical design experiments, estimation of coefficient using a mathematical model and an analysis of model applicability [11].

A five level, central composite design was used to evaluate the relationship between the culture conditions (independent variables) and the carbon fixation rate (dependent variable). Three replicates at the central point were used to estimate the experimental error.

The experimental design and the statistical analyses were carried out using the Statistica 7.0 software (Statsoft, USA). Table 1 shows the levels of the experimental variables used:

For a 3-factor system, the statistical model is defined by Eq. (1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

The process was optimised from an analysis of the experimental design initially proposed, carried out in triplicate.

The calculated cell biomass values were used to calculate the maximum cell concentration (X_m, mg L^{-1}), maximum specific growth rate (μ_{max}, h^{-1}) and generation time (t_g, h). The carbon fixation rate was calculated from the elemental analysis of the biomass, as shown in Eq. (2) [12]:

$$R_C = C_c \times \left(\frac{X_m - X_0}{t - t_0}\right) \times \left(\frac{M_{CO_2}}{M_c}\right) \quad (2)$$

2.4. Analytical methods

The cell concentration was evaluated gravimetrically by filtering a known volume of culture medium through a 0.45 μm filter and drying at 60 °C for 24 h. The measurements of light intensity incident on the reactor were carried out on the external column surface using a digital luximeter (Minipa MLM 1010). The temperature was controlled using thermostats, and measured using a polarographic sensor (Mettler Toledo InPro5000 series). The flow rates of the carbon dioxide, air and CO_2 enriched air were determined using rotameters (AFSG 100 Key Instruments). The elemental composition of the *Aphanothece microscopica Nägeli* cells was determined using a Perkin Elmer 2400 CHNS/O element analyser. Two-milligram samples of biomass were oxidised at 1000 °C and the resulting gases were determined using a thermal conductivity probe for carbon, nitrogen and hydrogen. The standard used was acetanilide, with a composition of 71.09% carbon, 11.84% oxygen, 6.71% hydrogen and 10.36% nitrogen.

Table 2
Kinetic parameters for *Aphanothece microscopica Nägeli*

Condition				Kinetic parameter			
X_1 (°C)	X_2 (klux)	X_3 (%)	μ_{max} (h^{-1})	R^2	t_g (h)	t_{log} (h)	X_m (mg L^{-1})
35.0	3	15	0.027	0.99	25.67	60	1672
35.0	9	15	0.034	0.94	20.38	72	3000
35.0	9	50	0.025	0.97	27.72	72	600
35.0	3	50	0.022	0.99	31.50	72	500
25.0	3	15	0.010	0.97	69.31	36	270
25.0	9	15	0.013	0.90	53.31	108	455
25.0	3	50	0.023	0.98	30.13	108	600
25.0	9	50	0.023	0.98	30.13	96	905
21.5	6	25	0.024	0.99	28.88	84	730
38.5	6	25	0.003	0.98	231.1	36	230
30.0	11	25	0.030	0.98	23.10	72	2300
30.0	0.96	25	0.008	0.94	86.64	72	360
30.0	6	62	0.023	0.98	30.13	72	1135
30.0	6	3	0.021	0.97	33.01	48	1600
30.0	6	25	0.028	0.99	24.75	108	1760

3. Results and discussion

3.1. Preliminary tests

Fig. 2 shows representative growth curves for the cyanobacterium *Aphanothece microscopica Nägeli* under the different photosynthetic culture conditions. A comparison of the growth curves shows the lack of an adaptation phase for this microorganism, reaching the logarithmic growth phase as from 12 h of cultivation, followed by a stationary phase, and, under some conditions, a declining phase. According to Guerrero et al. [13], the absence of an adaptation phase in microalgal growth curves is characteristic of culture media with high carbon and inorganic nutrient availability. For all the cultures, after a maximum residence time of 5 days, the growth curves already indicated characteristics of the stationary phase, the maximum cell densities being obtained in this period. Similar results were obtained by Yue and Chen [14] and Hsueh et al. [15] in the photosynthetic cultivation of the microalgae *Chlorella* ZY-1 and *Nannochlopsis oculta*, obtaining positive growth rates in residence times below 120 h.

Table 2 shows the growth kinetics of *Aphanothece microscopica Nägeli*. An analysis of these results shows that the maximum specific growth rates were obtained at 35 °C, 9 klux and 15%

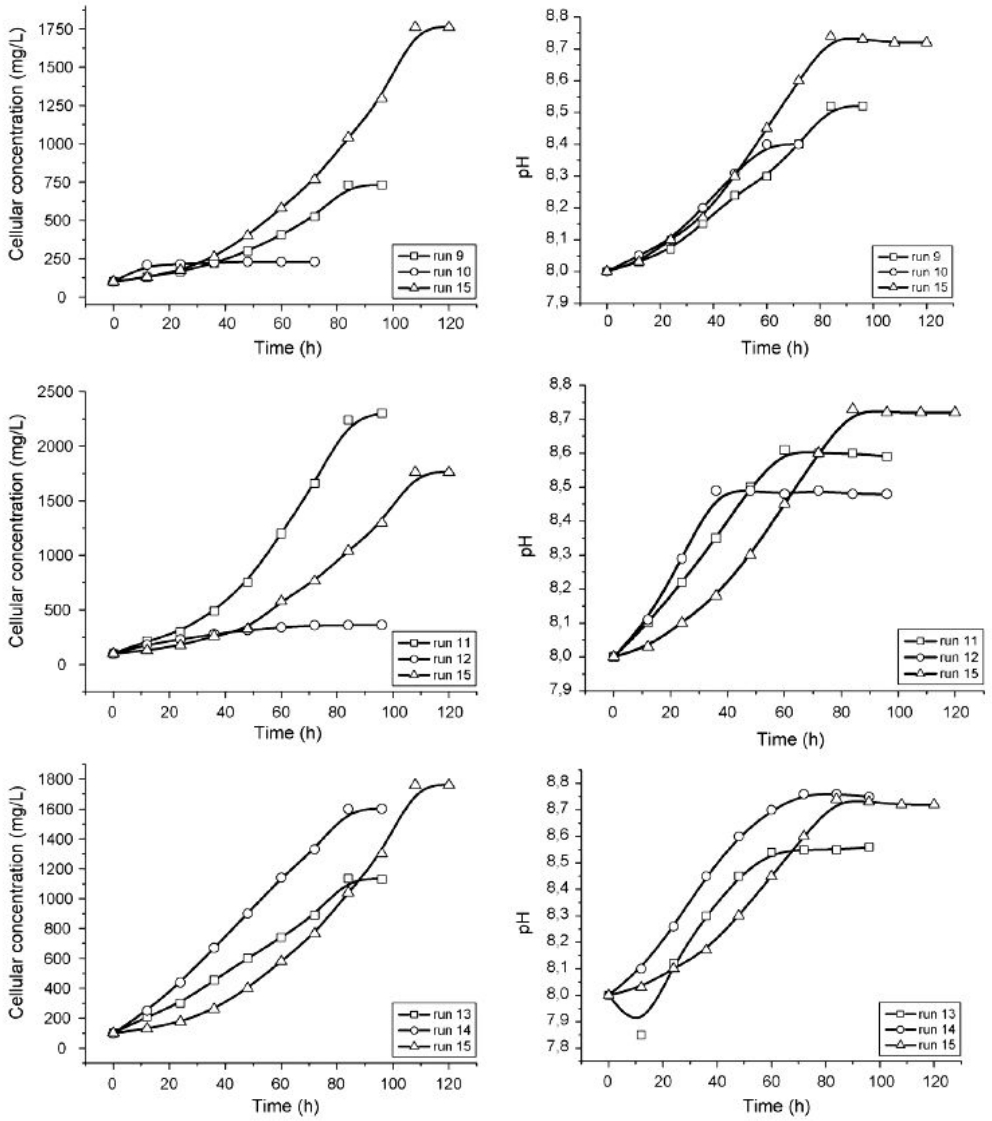

Fig. 2. Representative growth and pH curves as a function of temperature, light intensity and CO_2 concentration. Effect of temperature (run 9, 10 and 15); Effect of light intensity (run 11, 12 and 15) and effect of carbon dioxide concentration (run 13, 14 and 15).

CO_2, not differing significantly, according to the Tukey test ($p < 0.05$), from the cultures grown at 30 °C, 11 klux and 25% CO_2. The maximum values obtained for μ_{max} were similar to those obtained by other authors for the species *Euglena gracilis* and *Anabaena variabilis*, cultivated using photosynthetic metabolism [16,17]. However the maximum concentration of carbon dioxide enrichment used for these cultures was 13% (v/v), suggesting that the *Aphanothece microscopica Nägeli* is more tolerant to gases enriched with high levels of CO_2, since high growth rates were obtained with up to 62% (v/v) of carbon dioxide. With respect to the generation times, a variation between 23.1 and 231.1 h was observed, as a function of the different cultivation conditions, indicating a dependence of cell growth on the conditions of temperature, light intensity and concentration of carbon dioxide enriched air in the operation of the photobioreactor. The logarithmic growth phases, determined by linear regression of the data, indicated exponential growth between 35 and 108 h. The maximum cell density of 3.0 g L^{-1}, was obtained by cultivating in air enriched with 15% carbon dioxide at a temperature of 35 °C, with a light intensity of 9 klux. Of the conditions evaluated, the results suggest these experimental conditions as the most adequate to produce biomass by the cyanobacterium *Aphanothece microscopica Nägeli*.

Cell growth associated with carbon dioxide assimilation from the medium coincided with an increase in pH values. Fig. 2 shows the representative variation in pH of the culture medium as a function of residence time. The pH range observed in the culture medium varied from 6.0 to 9.9, indicating that the species HCO_3^- was the predominant form of carbon in the cultures.

The gradual increase in pH of the culture media, independent of the conditions, indicated that cultivation occurred in an alkaline pH range. Reductions in the initial pH value of the culture medium were only observed when cultivated with carbon dioxide concentrations equal or greater than 50%. On the other hand, the highest pH values (9.9) were found for the cultures with the highest cell densities (3.0 g L^{-1}), suggesting predominance of the photosynthetic metabolism of the microorganism under the experimental conditions used. According to Arnon [18] and Zuber [19], the increase in pH in photosynthetic cultures is an indication of the consumption of inorganic carbon due to cell growth. These authors reported that the increment in pH in the culture medium could be attributed to two main mechanisms: firstly the transport of hydroxide ions to outside the cell occurs by way of a reaction catalysed by the enzyme carbon anhydrase during the conversion of bicarbonate ions inside the cell to provide CO_2 for the photosynthetic reaction, raising the pH of the culture medium. A second potential mechanism would be the increase in pH due to activity of the enzyme ribulose 1,5-bisphosphate carboxylase, whose activity is considerably dependent on pH, increasing with increase in pH. This enzyme is present in the photosynthetic apparatus of the cyanobacteria, where the H^+ ions are sequestered to the inside of the tylacoid membrane with a simultaneous transfer of Mg^{2+} to the environment. These light energy induced fluxes result in an increase in pH and in the Mg^{2+} concentration, activating the RubisCO enzyme and resulting in efficient carbon dioxide fixation.

An analysis of the elements in the *Aphanothece microscopica Nägeli* cells at the end of cultivation showed that 1 g of biomass could contain between 0.48 and 0.52 g of carbon as a function of the cultivation conditions used. These values were used to estimate the carbon fixation rates (Table 3).

Table 4 shows the results for the effects of and the interactions between the factors of temperature, light intensity and carbon dioxide concentration, as also the coefficients of the model. An analysis of this table shows that in the range evaluated, the carbon fixation rate was controlled by the factors of light intensity (L), temperature (Q), interaction between temperature (L) and carbon dioxide concentration (L), and by the carbon dioxide concentration (L), in this order of importance. The other factors and interactions showed a lower proportion of statistical importance.

Table 3
Coded matrix of the effects of temperature, light intensity and CO_2 concentration on the carbon fixation rate

Run	Temperature (X_1)	Light intensity (X_2)	CO_2 (X_3)	R_C (mg L^{-1} h^{-1})
1	+1	−1	−1	24.80
2	+1	+1	−1	45.78
3	+1	+1	+1	11.84
4	+1	−1	+1	11.36
5	−1	−1	−1	3.45
6	−1	+1	−1	6.30
7	−1	−1	+1	8.88
8	−1	+1	+1	16.30
9	−1.68	0	0	12.78
10	+1.68	0	0	4.61
11	0	+1.68	0	39.07
12	0	−1.68	0	6.15
13	0	0	+1.68	21.01
14	0	0	−1.68	30.44
15	0	0	0	26.20
16	0	0	0	25.96
17	0	0	0	26.04

Table 4
Coefficients of the model estimated by linear regression

Factor	Effects	Standard error	$t(2)$	p	Coefficients	Estimates per interval	
						−95%	+95%
Mean	26.22	0.07	372.47	0.00000	26.22	25.92	26.53
X_1 (L)	6.60	0.06	99.88	0.00010	3.30	3.16	3.44
X_1 (Q)	−13.39	0.07	−183.95	0.00003	−6.69	−6.85	−6.53
X_2 (L)	12.75	0.06	192.85	0.00002	6.37	6.23	6.51
X_2 (Q)	−3.55	0.07	−48.78	0.00042	−1.77	−1.93	−1.61
X_3 (L)	−7.00	0.06	−105.86	0.00008	−3.50	−3.64	−3.35
X_3 (Q)	−1.34	0.07	−18.52	0.00290	−0.67	−0.83	−0.51
X_1(L) × X_2(L)	2.79	0.08	32.37	0.00095	1.39	1.21	1.58
X_1(L) × X_3(L)	−15.70	0.08	−181.72	0.00003	−7.85	−8.03	−7.66
X_2(L) × X_3(L)	−3.98	0.08	−46.08	0.00047	−1.99	−2.17	−1.80

Eq. (3) represents the statistical model for the variable response carbon fixation rate:

$$Y = 26.2 + 3.3X_1 - 6.7X_1^2 + 6.4X_2 - 1.77X_2^2 - 3.5X_3 - 0.67X_3^2 + 1.4X_1X_2 - 7.85X_1X_3 - 2.0X_2X_3 \quad (3)$$

Contour curves (Fig. 3) show the variation in the carbon fixation rate as a function of the factors studied. Thus an increase in carbon fixation rate is obtained not only by fixing the light intensity at the highest level, that is, carrying out the cultivation under high light intensities, but also by fixing the temperature in the central region and the carbon dioxide concentration at the lowest level (reduced proportions of CO_2 enrichment in the air entering the photobioreactor).

The carbon fixation rate varied from 3.45 to 45.78 mg L^{-1} h^{-1} under the different culture conditions. These results are higher than those obtained by Kajiwara et al. [12] when cultivating the cyanobacterium *Synechoccus* (PCC 7942) in photobioreactors at 30 °C with 8 klux and air enriched with carbon dioxide at 5%, obtaining maximum carbon fixation rates to the order of 25 mg L^{-1} h^{-1} and Yun et al. [20], who obtained rates of 23.4 mg L^{-1} h^{-1} for the microalga *Chlorella vulgaris* (UTEX 259) under conditions of 27 °C, 15% of CO_2 and 110 μE m^{-2} s^{-1}.

These results reflect the adaptation of the microorganism under study to the experimental conditions, suggesting the importance of optimising the photobioreactor operational conditions in order to obtain higher rates of biological carbon fixation.

Different authors have reported the importance of the environmental conditions on the growth of cyanobacteria. Parameters such as temperature, light intensity, pH and carbon dioxide concentration have been indicated as being of fundamental importance in the development of these microorganisms [21]. Thus it was shown that cultivation conditions in the temperature range from 30 to 35 °C, with 9–11 klux and 15–25% CO_2, favoured cell growth, associated with carbon fixation. These results agreed with those of Munoz and Guieysse [22], who reported that the efficiency of systems using microalgae normally decreased at low and higher temperatures, justifying the low cell performance of the cultures incubated at 21.5 and 38.5 °C, when compared to those incubated at 30 °C under the same conditions of light intensity and CO_2 concentration. According to Grossman et al. [23], the optimum temperature range for the development of cyanobacteria is from 25 to 35 °C.

With respect to light intensity, it was shown that the maximum intensity used was insufficient to promote the phenomenon of cell photoinhibition, since even at 11 klux high growth rates were recorded. On the other hand, at 0.96 klux there was a pronounced reduction in cell growth, suggesting limitation of the energy required for the sequence of photosynthetic reactions that would result in carbon fixation under these conditions.

The effect of different carbon dioxide concentrations in the gases entering the photobioreactor was analysed by Yue and Chen [14], who found photosynthetic activity for concentrations of up to 70% (v/v) enrichment of the air with CO_2, which is usually the main source of carbon in photosynthetic cultures of cyanobacteria. According to Cuaresma et al. [24], the way in which cyanobacteria adapt themselves to use CO_2 as the carbon source is related to the carbon concentration mechanisms (CCM). The photosynthetic mechanism developed

Table 5
Analysis of variance (ANOVA) for the quadratic model

Source of variation	Sum of squares	Degrees of freedom	Mean squared	$F_{calculated}$
Regression	1938.8	9	215.42	2.83[a]
Residues	532.66	7	76.09	
Lack of fit	532.63	5	106.52	
Pure error	0.03	2	0.015	
Total	2741.44	16		

[a] Statistical significance ($p < 0.1$).

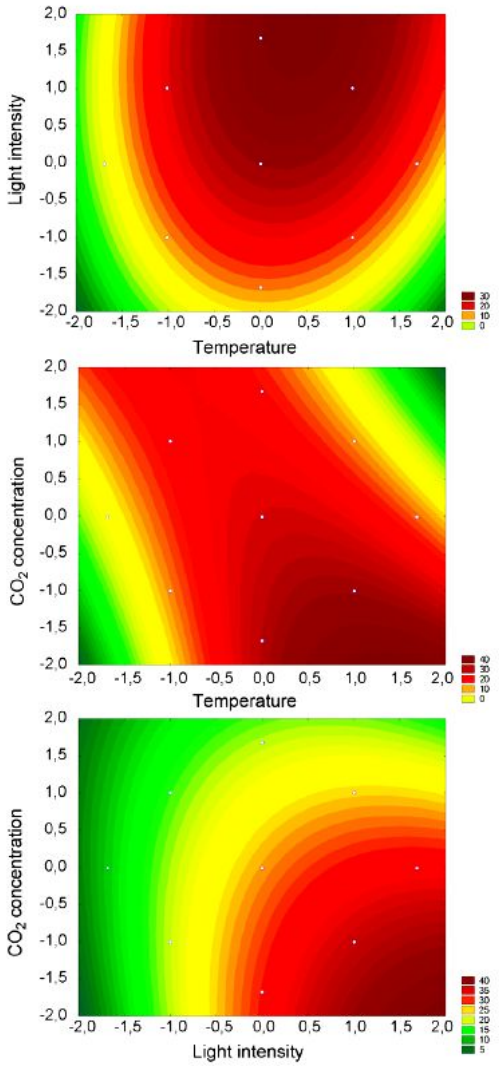

Fig. 3. Contour curves for the variable carbon fixation rate.

by these organisms allowed for the transport of free CO_2 and of bicarbonate ions through a fine plasmatic membrane, being accumulated in the cell as a carbon reservoir, making it possible to assimilate inorganic carbon in the form of bicarbonate or free carbon dioxide. With respect to cell growth and carbon fixation rate, the best results were obtained with air enriched with 15% CO_2, reduced efficiency of the photobioreactor being observed with flow rates below or above 0.45 L_{CO2} min^{-1}.

The model was validated from the F distribution (Table 5), which suggested the existence of a quadratic relationship between the variables, indicating that the proposed model fitted the experimental data. The statistical model obtained explained a maximum of 99.99% of the variation.

Table 6
Kinetic parameters for process optimization

Kinetic variable	Value[a]
μ_{max} (h^{-1})	0.04
t_g (h)	17.3
t_{log} (h)	120
X_m (mg L^{-1})	5100
R_C (mg L^{-1} h^{-1})	109.2

[a] Mean of three replicates are shown.

3.2. Process optimisation

As from the statistical model and an analysis of the contour diagrams, which suggested a displacement of the operational conditions to the region of high light intensities (11 klux), maintaining the conditions of temperature at 35 °C and injection of air enriched with 15% CO_2, cultures were carried out under these conditions in order to optimise the fixation of carbon by the cyanobacterium *Aphanothece microscopica Nägeli*. Table 6 shows the kinetic parameters obtained for the process under these conditions. A significant increase in the kinetic variables of growth and carbon fixation was observed, obtaining carbon fixation rates 58.1% higher than those obtained under the best conditions initially evaluated (9 klux, 35 °C, 15% of CO_2), showing the importance of the factors studied and the adjustment of the methodology used.

4. Conclusions

The carbon dioxide concentration, temperature and light intensity are determinant factors in the carbon fixation process by the cyanobacterium *Aphanothece microscopica Nägeli*.

Under operational conditions of 11 klux, 35 °C and 15% CO_2, the microorganism performed better, obtained maximum specific growth rates and carbon fixation rates of 0.04 h^{-1} and 109.2 mg L^{-1} h^{-1}, respectively.

Response surface methodology was adequate to determine the effects of the factors and optimise the process.

Thus the results obtained in the present study suggest the potential of applying this type of process to obtain carbon credits. Nevertheless one must remember that the definition of the ideal process conditions only helps in terms of the magnitude of the variables involved in the system, with the objective of applying the process under complex real conditions. In addition, the carbon fixed in the biomass only represents a fraction of the total carbon dioxide transformed by the system.

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References

[1] H. Yamada, N. Ohkuni, S. Kajiwara, K. Ohtaguchi, CO_2-removal characteristics of *Anacystis nidulans* R2 in airlift bioreactors, Energy 22 (1997) 349–352.

[2] H.W. Yen, D.E. Brune, Anaerobic co-digestion of algal sludge and waste paper to produce methane, Bioresour. Technol. 98 (2007) 130–134.

[3] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294–306.

[4] B.D. Lee, W.A. Apel, M.R. Walton, Calcium carbonate formation by *Synechococcus* sp. strain PCC 8806 and *Synechococcus* sp. strain PCC 8807, Bioresour. Technol. 97 (2006) 2427–2434.

[5] E. Jacob-Lopes, C.H.G. Scoparo, T.T. Franco, Rates of CO_2 removal by *Aphanothece microscopica Nägeli* in tubular photobioreactors, Chem. Eng. Process., doi:10.1016/j.cep.2007.06.004.

[6] F.A. Esteves, in: Rio de Janeiro (Ed.), Fundamentos de limnologia, Internacional, 1988.

[7] E. Jacob-Lopes, L.Q. Zepka, L.A.A. Pinto, M.I. Queiroz, Characteristics of thin-layer drying of the cyanobacterium *Aphanothece microscopica Nägeli*, Chem. Eng. Process. 46 (2006) 63–69.

[8] M.I. Queiroz, E. Jacob-Lopes, L.Q. Zepka, R. Bastos, R. Goldbeck, The kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor, Bioresour. Technol. 98 (2007) 2163–2169.

[9] L.Q. Zepka, E. Jacob-Lopes, R. Goldbeck, M.I. Queiroz, Production and biochemical profile of the microalgae *Aphanothece microscopica Nägeli* submitted to different drying conditions, Chem, Eng, Process., doi:10.1016/j.cep.2007.04.013.

[10] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic assignments strain histories and properties of pure cultures of cyanobacteria, J. Gen. Microbiol. 111 (1979) 1–61.

[11] G.E.P. Box, W.G. Hunter, J.S. Hunter, Statistics for Experiments, John Wiley and Sons, New York, 1978.

[12] S. Kajiwara, H. Yamada, O. Narumasa, Design of the bioreactor for carbon dioxide fixation by *Synechococcus* PCC7942, Energy Conv. Manage. 38 (1997) 529–532.

[13] L. Guerrero, F. Omil, R. Mendez, J.M. Lema, Anaerobic hydrolysis and acidogenesis of wastewaters from food industries with high content of organic solids and protein, Water Res. 33 (15) (1999) 3250–3281.

[14] L. Yue, W. Chen, Isolation and determination of cultural characteristics of a new highly CO_2 tolerant fresh water microalgae, Energy Conv. Manage. 46 (2005) 1846–1896.

[15] H.T. Hsueh, H. Chu, S.T. Yu, A batch study on the bio-fixation of carbon dioxide in the absorbed solution from a chemical wet scrubber by hot springs and marine algae, Chemosphere 66 (2007) 878–886.

[16] S.R. Chae, E.J. Hwang, H.S. Shin, Single cell protein production of *Euglena gracilis* and carbon dioxide fixation in an innovative photo-bioreactor, Bioresour. Technol. 97 (2006) 322–329.

[17] J.H. Yoon, High cell density culture of *Anabaena variabilis* using repeated injections of carbon dioxide for the production of hydrogen, Int. J. Hydrogen Energy 27 (2002) 1265–1270.

[18] H. Zuber, Structure of light-harvesting antenna complexes of photosynthetic bacteria, cyanobacteria and red algae, Trends Biochem. Sci. 11 (1986) 414–419.

[19] D.I. Arnon, The discovery of photosynthetic phosphorylation, Trends Biochem. Sci. 9 (1984) 258–262.

[20] Y.S. Yun, S.B. Lee, J.M. Park, C. Lee, J. Yang, Carbon dioxide fixation by algal cultivation using wastewater nutrients, J. Chem. Technol. Biotechnol. 69 (1997) 451–455.

[21] G. Subramanian, N. Thajuddin, Cyanobacterial biodiversity and potential applications in biotechnology, Curr. Sci. 89 (2005) 47–57.

[22] R. Muñoz, B. Guieysse, Algal-bacterial processes for the treatment of hazardous contaminants: a review, Water Res. 40 (2006) 2799–2815.

[23] A.R. Grossman, M.R. Schaefer, G.G. Chiang, J.L. Collier, The responses of cyanobacteria to environmental conditions: light and nutrients, in: D.A. Bryant (Ed.), The Molecular Biology of cyanobacteria, Kluwer Academic Plubishers, 1994, pp. 641–668.

[24] M. Cuaresma, I. Garbayo, J.M. Vega, C. Vílchez, Growth and photosynthetic utilization of inorganic carbon of the microalga *Chlamydomonas acidophila* isolated from Tinto river, Enzyme Microb. Technol. 40 (2006) 158–162.

ANEXO II

Effect of light cycles (night/day) on CO2 fixation and biomass production by microalgae in photobioreactors

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Effect of light cycles (night/day) on CO_2 fixation and biomass production by microalgae in photobioreactors

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ABSTRACT

The objective of this study was to evaluate the effect of the photoperiod on the biomass production and carbon dioxide fixation rates using a photosynthetic culture of the cyanobacterium *Aphanothece microscopica Nägeli* in bubble column photobioreactors. The cultures were carried out at temperatures of 35 °C, air enriched with carbon dioxide at concentrations of 15% and photon flux density of 150 μmol m^{-2} s^{-1}. The light cycles evaluated were 0:24, 2:22, 4:20, 6:18, 8:16, 10:14, 12:12, 14:10, 16:8, 18:6, 20:4, 22:2 and 24:0 (night:day), respectively. The results obtained indicated that the duration of the light periods was a determinant factor in the performance of the photobioreactors. A linear reduction in biomass production and carbon dioxide fixation with reductions in the duration of the light period was evident, with the exception of the 12:12 (night:day) cycles. Reductions of up to 99.69% in the carbon-fixation rates as compared with cultures under continuous illumination were obtained.

1. Introduction

Various research projects are underway involving the application of cyanobacteria and microalgae in carbon dioxide biofixation, aiming to project viable photobioreactors for CO_2 sequestration technology [1–3].

Photosynthesis is a process comprising two steps, light reactions that only occur when the cells are illuminated, and carbon-fixation reactions, also known as dark reactions, that occur both in the presence and absence of light. Thus in the first step the cells transform light energy into chemical energy, which is stored in high-energy compounds for later use in the carbon-fixation reactions [4].

The use of these photosynthetic pathways in environmental engineering processes requires the use of solar energy so as to develop clean technology processes [5]. Thus the cells use the light energy by way of exergonic reactions, producing energy that is used in the synthesis of compounds as from carbon dioxide fixation by way of endergonic reactions [6]. However, one of the operational problems of this type of technology refers to the lack of availability of light energy for whole time periods.

The light regimes to which the cultures are submitted are considered to be an important factor in the productivity and yield of photosynthetic reactions [7,8]. Various studies have been carried out focused on the effect caused by different photon flux densities incident on photobioreactors, but few reports can be found on the effects of the duration of the day and night cycles [9–11]. Thus a comparison of different photoperiods is necessary in order to determine the most efficient light regimes for industrial purposes.

Thus the objectives of the present study were to evaluate the effect of the photoperiod on biomass production and carbon dioxide fixation rates by the cyanobacterium *Aphanothece microscopica Nägeli* in bubble column photobioreactors.

2. Materials and methods

2.1. Microorganism and culture medium

Unialgal cultures of *Aphanothece microscopica Nägeli* (RSMan92) were originally isolated from the Patos Lagoon estuary, Rio Grande do Sul State, Brazil (32°01′S–52°05′W). Stock cultures were propagated and maintained on synthetic BGN medium [12], with the following composition (g/L): $K_2HPO_4{\cdot}3H_2O$ (0.040), $MgSO_4{\cdot}7H_2O$ (0.075), EDTA (0.001), H_3BO_3 (2.860), $MnCl_2{\cdot}4H_2O$ (1.810), $ZnSO_4{\cdot}7H_2O$ (0.222), $Na_2MoO_4{\cdot}2H_2O$ (0.390), $CuSO_4{\cdot}5H_2O$ (0.079), $CaCl_2{\cdot}6H_2O$ (0.040), $NaNO_3$ (150), $C_6H_8O_7{\cdot}H_2O$ (0.006), ammonium iron citrate (0.006), pH 8.0. The incubation conditions used were 25 °C, photon flux density of 15 μmol m^{-2} s^{-1} and a photoperiod of 12 h.

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2.2. Photobioreactor design

Measurements were made in a bubble column photobioreactor. The system was built in 4-mm thick glass, an internal diameter of 7.5 cm, height of 75 cm and nominal working volume of 3.0 L. The dispersion system for the reactor consisted of a 1.5 cm diameter air diffuser located in the centre of the column. The reactor was continuously illuminated with sixteen 20 W fluorescent daylight-type tubes (General Electric, Brazil) connected in parallel, located in a photoperiod chamber. The duration of light cycles was controlled by timer. Airflow into the photobioreactor was provided via filtered air and pure CO_2 cylinder through Teflon tubing. The CO_2/air mixture was adjusted to achieve the desired concentration of carbon dioxide in the airstream, through three rotameters that measured the flow rates of the carbon dioxide, the air and the mixture of gases, respectively.

2.3. Obtaining of the kinetic data in an experimental photobioreactor

The experiments were carried out in bioreactors operating in a batch mode, fed with 3.0 L synthetic BGN medium. The experimental conditions were the following: initial cell concentration of 0.1 g L^{-1}, isothermal reactor operating at a temperature of 35 °C, photon flux density of 150 μmol m^{-2} s^{-1} and continuous aeration of 1 VVM with the injection of air enriched with 15% carbon dioxide. Such conditions were previously defined by Jacob-Lopes et al. [13]. The light cycles evaluated were 0:24, 2:22, 4:20, 6:18, 8:16, 10:14, 12:12, 14:10, 16:8, 18:6, 20:4, 22:2 and 24:0 (night:day), respectively. The cell concentration and the carbon-fixation rate were monitored every 12 h during the growth phase of the microorganism. The tests were carried out in duplicate and the kinetic data referred to the mean of four repetitions.

2.4. Kinetic parameters

The volumetric growth rate was determined from the variation in cell concentration in a determined time interval, as shown in Eq. (1):

$$P_X = \frac{X_1 - X_0}{t_1 - t_0} \quad (1)$$

where X_0 and X_1 are the biomass concentration at times t_0 and t_1, respectively. In the present study, t_0 and t_1 were zero and 156 h, respectively. In light cycles of 24:0 (night:day), the t_1 considered was 48 h.

The carbon dioxide fixation rate was evaluated from the relationship between the carbon content of the cells and the volumetric growth rate of the microorganism, as shown in Eq. (2):

$$R_C = C_C P_X \left(\frac{M_{CO_2}}{M_C} \right) \quad (2)$$

2.5. Analytical methods

The cell concentration was evaluated gravimetrically by filtering a known volume of culture medium through a 0.45 μm filter and drying at 60 °C for 24 h. The photon flux density was determined using a digital photometer (Spectronics, model XRP3000), measuring the light incident on the external reactor surface. The temperature was controlled using thermostats, and measured using a polarographic probe (Mettler Toledo, InPro5000 series). The flow rates of the carbon dioxide, air and CO_2 enriched air were determined using rotameters (AFSG 100 Key Instruments). The composition of the elements of the *Aphanothece microscopica Nägeli* cells was determined using a PerkinElmer 2400 CHNS/O element analyser. Two milligrams sample of biomass were oxidised at 1000 °C and the resulting gases were determined using a thermal conductivity probe for carbon. The standard used was acetanilide, with a composition of 71.09% carbon, 11.84% oxygen, 6.71% hydrogen and 10.36% nitrogen.

3. Results and discussion

In photosynthetic cultures, the amount of light energy received and stored by the cells has a direct relationship with the carbon-fixation capacity, consequently determining the productivity in biomass and cell growth rate. In nature, light energy is available in a discontinuous way, since the light varies from day to night. Such considerations are relevant in carbon sequestration processes in photobioreactors, since the viability of these systems requires the use of solar energy for photosynthesis. Systems of this type, fundamentally based on natural resources, are highly affected by the lack of availability of light energy during whole periods of time. Fig. 1 shows the variation in growth of the cyanobacterium *Aphanothece microscopica Nägeli* under conditions of 35 °C, 150 μmol m^{-2} s^{-1} and 15% CO_2 with a continuous source of light energy.

An analysis of the growth curve shows the lack of an adaptation phase for the microorganism, with exponential growth occurring as from 12 h of cell residence time. The stationary phase was observed as from the sixth day of culture. The maximum cell concentrations obtained were 5.100 g/L, representing a more than 50-fold increase in cell density as compared to that initially present in the reactor.

Fig. 2 shows the growth curves for the cyanobacterium *Aphanothece microscopica Nägeli*, with light cycles reduced at 2 h intervals.

Different cell growth profiles can be seen as a function of the duration of the light periods. The cultures grown under photoperiods of 2:22 (night:day) showed characteristics similar to those grown with a continuous supply of light energy, whilst those grown in the absence of light showed evidence of limited carbon source for cell growth, since the cyanobacteria are unable to use inorganic carbon sources in the absence of light, and the organic carbon concentrations in the culture medium were insufficient for the energy maintenance of respiratory metabolism [14].

These results are more evident in Table 1, which presents the kinetic characterisation of the growth and carbon fixation in the biomass by *Aphanothece microscopica Nägeli*, under the different light cycles evaluated.

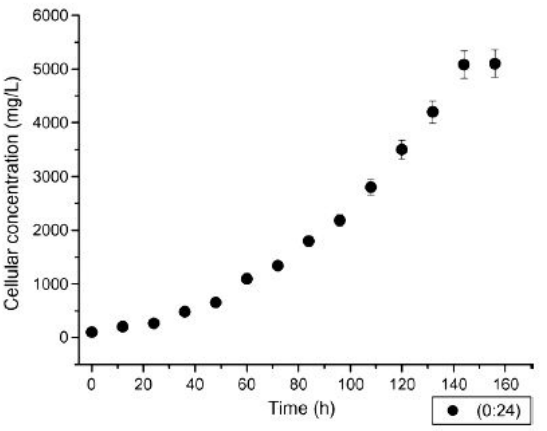

Fig. 1. Growth curve under a continuous light regime (dark:light).

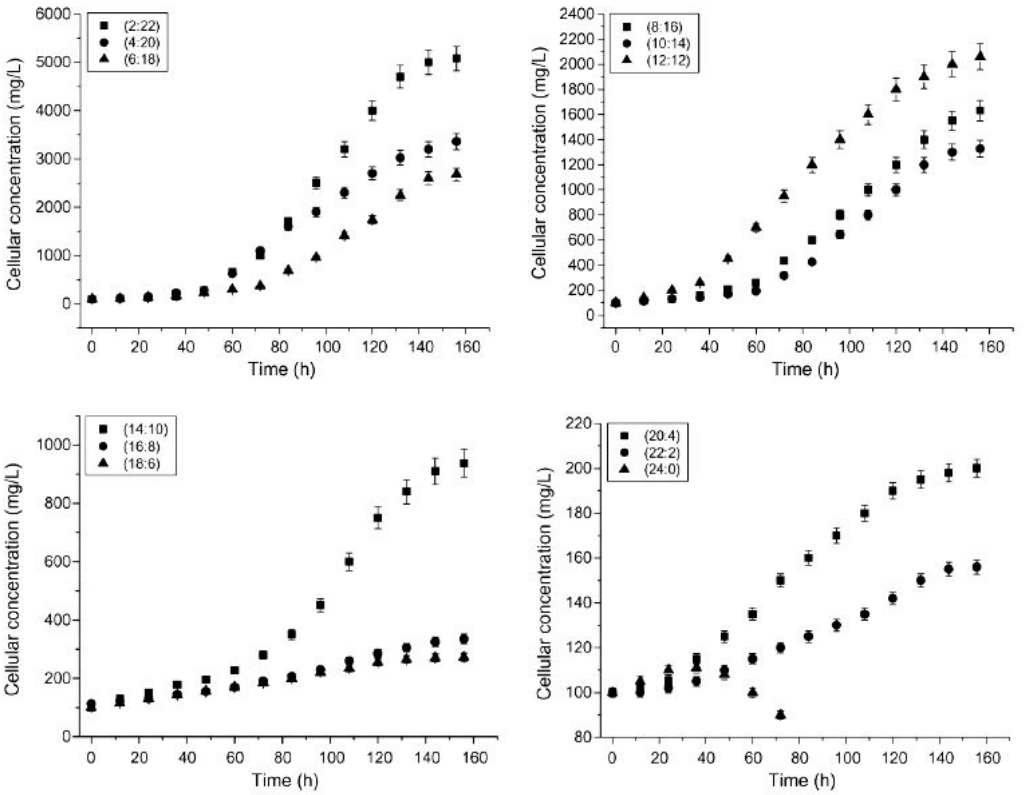

Fig. 2. Growth curves in different light cycles (dark:light).

From the analysis of variance (ANOVA) and Tukey's test ($p < 0.05$), maximum volumetric productivity was shown to occur under conditions with a constant supply of light energy (0:24), the values not differing significantly ($p = 0.41$) from those obtained in experiments carried out with photoperiods of (2:22), suggesting that the supply of light for periods greater than 22 h did not influence the volumetric growth rate. These results demonstrate that *Aphanothece microscopica Nägeli* is capable of storing sufficient energy to sustain cell growth for periods of up to a maximum of 2 h in the dark, without affecting the rate of photosynthetic metabolism. For the other photoperiods evaluated, all the values for volumetric growth rate differed statistically ($p < 0.0001$). Similar results were obtained for the maximum cell densities, for which the photoperiods (0:24) and (2:22) (night:day) were statistically equal ($p < 0.05$). On the other hand, it was shown there were no significant differences in the maximum cell concentrations for the cultures grown with dark periods greater than 18 h.

Table 1
Kinetic parameters for *Aphanothece microscopica Nägeli* in different light cycles

Photoperiod (night/day) (h)	P_X (g/L day)	X_{max} (g/L)	R_{CO_2} (g/L day)
0:24	$0.770^{a} \pm 0.038$	$5.100^{a} \pm 0.255$	$1.440^{a} \pm 0.072$
2:22	$0.764^{a} \pm 0.042$	$5.080^{a} \pm 0.305$	$1.428^{a} \pm 0.085$
4:20	$0.501^{b} \pm 0.025$	$3.400^{b} \pm 0.187$	$0.936^{b} \pm 0.065$
6:18	$0.235^{c} \pm 0.014$	$2.685^{c} \pm 0.174$	$0.439^{c} \pm 0.032$
8:16	$0.240^{d} \pm 0.016$	$1.640^{d} \pm 0.116$	$0.448^{c} \pm 0.040$
10:14	$0.189^{e} \pm 0.009$	$1.300^{e} \pm 0.052$	$0.353^{d} \pm 0.021$
12:12	$0.301^{f} \pm 0.016$	$2.060^{f} \pm 0.072$	$0.562^{e} \pm 0.025$
14:10	$0.127^{g} \pm 0,006$	$0.944^{g} \pm 0.018$	$0.237^{f} \pm 0.014$
16:8	$0.035^{h} \pm 0,002$	$0.343^{h} \pm 0.013$	$0.065^{g} \pm 0.003$
18:6	$0.026^{i} \pm 0.001$	$0.260^{i} \pm 0.013$	$0.048^{g} \pm 0.003$
20:4	$0.015^{j} \pm 0.000$	$0.200^{i} \pm 0.017$	$0.028^{g} \pm 0.001$
22:2	$0.008^{k} \pm 0.000$	$0.150^{i} \pm 0.009$	$0.015^{g} \pm 0.001$
24:0	$0.002^{l} \pm 0.000$	$0.110^{i} \pm 0.004$	$0.004^{g} \pm 0.000$

Values are mean ± S.D. of quadruplicate analysis; Within the same column, means having different superscripts (a–l) are significantly different ($p < 0.05$) by Tukey's test.

The influence of the light cycles has been reported as a determinant factor in photosynthetic activity and in the growth rates of microalgae in photobioreactors [15–17]. According to these authors, light is a limiting substrate in these systems, which are affected by light/dark zones that depend primarily on the configuration, agitation and mixture in the reactor, associated with the possibility of cultures with discontinuous periods of light energy supply. Additionally, cell concentration is another parameter which determines the availability of light in photobioreactors. As result of the mutual shading occurring at high cell densities, the light intensity within the reactor becomes also a function of the biomass concentration [18]. As a result, the cells are exposed to different light intensities, with a considerable effect on system performance.

The pronounced variations in the volumetric growth rates and maximum cell density as a function of the duration of the light cycle showed that the cell concentration decreased proportionally with the fraction of time that the microorganism was exposed to intermittent light conditions as compared to continuous illumination. An exception to this behaviour was shown with

photoperiods of 12:12 (night:day), in which higher productivity (0.301 g/L day) and maximum cell density values (2.060 g/L) were obtained than for photoperiods with 14h (0.189 g/L day, 1.300 g/L) and 16 h (0.240 g/L day, 1.640 g/L) of light.

These results are related to the fact that the cultures were maintained and propagated under a 12 h light cycle, resulting in an improvement in the volumetric growth rate and maximum cell concentrations under these conditions. Sicko-Goad and Andresen [8] obtained similar results, reporting that some species of microalgae could show preferences with respect to the duration of the light periods, resulting from the environmental conditions in which they were isolated in nature. Grobbelaar et al. [19] corroborated these results, reporting that independent of culturing under continuous or intermittent light conditions, acclimatisation of the cultures is determinant in the photosynthetic rates of the microalgae. On the other hand, Toro [7], comparing the growth of the microalgae *Chaetoceros gracilis* and *Isochrysis galbana* under culture conditions with photoperiods of 0:24 and 12:12 (night:day), respectively, supplied the cultures grown with photoperiods of 12:12 with double the light intensity during the light periods as compared to the cultures receiving continuous light energy, obtaining equal growth rates, suggesting that cell growth is also affected by the amount of energy offered per cycle, and not only by the duration of the photoperiod.

These results suggest the possibility of storing light energy by way of exergonic reactions, supplying an excess of energy for later use in subsequent endergonic reactions that can occur in periods in which there is an absence of light. From the operational point of view, this situation would be technologically interesting for use in photobioreactors, in which species capable of storing substantial amounts of light energy would show better performance in the application of this type of process. However, the limited capacity of the majority of microalgae to store light energy, means that in these cases the majority of the energy is dissipated in the reactors in the form of heat [15].

The carbon dioxide fixation rates are associated with the energy received by the cells during the light periods. Maximum carbon dioxide fixation rates of 1.440 g/L day were found for cultures with a continuous supply of light energy. A linear reduction in the CO_2 fixation rates with the reduction in duration of the light period was evident, with the exception of the 12:12 (night:day) cycles. It was also observed that carbon dioxide fixation did not differ significantly ($p < 0.05$) in cultures grown with dark periods greater than 16 h.

Fig. 3 shows the distribution of the percent carbon dioxide fixed in the biomass under the different conditions evaluated, the reference being the experiment carried out with a continuous light supply. An analysis of the diagram shows that the discontinuous supply of light resulted in a reduction in the CO_2 fixation rate between 2.0 and 99.69%, indicating the importance of the light phase of photosynthesis in the subsequent carbon-fixation reactions.

Besides the changes in the rates of biomass production and CO_2 fixation, one should also consider that the biomass formed in each photoperiod condition might have a different biochemical composition. Microalgae cells cultivated photoautotrophically under limited light conditions preferentially assimilate carbon in the direction of the synthesis of amino acids and other essential cell constituents, but under saturated light conditions, sugars and starch are formed via the pentose phosphate-reducing pathway [20,21], suggesting the dependence of the biomass composition with the light availability.

So the results obtained for the different light cycles evaluated indicated that the development of technology for the biological fixation of carbon dioxide in photobioreactors depended fundamentally on the access to light energy, which should be provided by solar energy so as to develop clean technology processes. Thus this type of CO_2 sequestration process could be very efficient in tropical countries, especially in locations near the equator where the light and dark periods are very similar and are associated with favourable temperature conditions. Martins et al. [22] reported that countries like Brazil show an ample potential for the exploration of solar energy, with mean solar irradiation varying from 4.25 to 6.50 kW/m^2 day in the different regions included in its territory. According to these authors, all regions in Brazil receive elevated light energy indices, with the potential for use as an energy source.

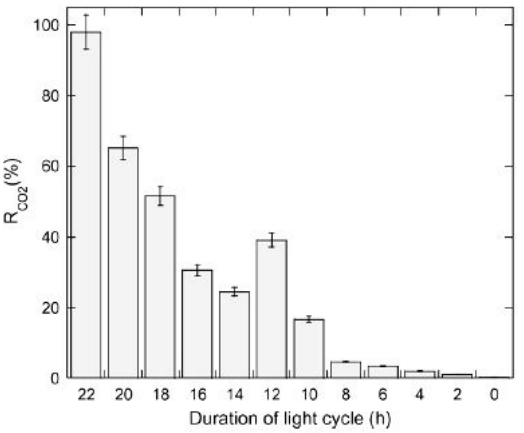

Fig. 3. Percent carbon fixation as related to the duration of the light periods.

4. Conclusions

The development of photobioreactors for carbon dioxide sequestration is a potential technology for application in tropical countries with elevated solar light availability. However, in order to predict the real carbon dioxide removal rates and biomass production in such systems, the lack of availability of light energy for part of the time during complete 24-h time periods must be considered, and this was evident from the cultivation of the cyanobacterium *Aphanothece microscopica Nägeli* under different photoperiod conditions.

Maximum values of 0.770 $g_{biomass}$/L day, 5.100 $g_{biomass}$/L and 1.440 g_{CO_2}/L day were obtained for volumetric productivity, maximum cell concentration and carbon dioxide fixation rate, respectively, under continuous light regime.

Linear reduction of the performance of the microorganism was evidenced in function of the duration of light regime. Exception to this behaviour was obtained in light cycles of the 12:12 (dark/light), suggesting the importance of the pre-adaptation of the microalgae cultures.

Thus the results obtained in the present study suggest the potential of applying this type of process to remove carbon dioxide. Nevertheless, the duration of the light cycles (night:day) is one fundamental criteria which should therefore be considered when projecting and analysing photobioreactors for the sequestering of carbon dioxide and biomass production.

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Appendix A. Nomenclature

C_C	Percent carbon in the biomass (%)
M_C	molecular weight of carbon
M_{CO_2}	molecular weight of CO_2
P_X	volumetric growth rate (g/L day)
R_{CO_2}	carbon dioxide fixation rate (g/L day)
t_0	cell residence time in $t = 0$ (h)
t_1	cell residence time in $t = n$ (h)
X_0	cell concentration in $t = 0$ (g/L)
X_1	cell concentration in $t = n$ (g/L)
X_{max}	maximum cell density (g/L)

References

[1] L. Yue, W. Chen, Isolation and determination of cultural characteristics of a new highly CO_2 tolerant fresh water microalgae, Energy Convers. Manage. 46 (2005) 1846–1896.
[2] B.D. Lee, W.A. Apel, M.R. Walton, Calcium carbonate formation by *Synechococcus* sp. strain PCC 8806 and *Synechococcus* sp. strain PCC 8807, Bioresour. Technol. 97 (2006) 2427–2434.
[3] E. Jacob-Lopes, C.H.G. Scoparo, T.T. Franco, Rates of CO2 removal by Aphanothece microscopica Nägeli in tubular photobioreactors, Chem. Eng. Process. 47 (2008) 1371–1379.
[4] T.M. Iverson, Evolution and unique bioenergetic mechanisms in oxygenic photosynthesis, Curr. Opin. Chem. Biol. 10 (2006) 91–100.
[5] T. Essam, M.A. Amin, O.E. Tayeb, B. Mattiasson, B. Guieysse, Solar-based detoxification of phenol and *p*-nitrophenol by sequential TiO_2 photocatalysis and photosynthetically aerated biological treatment, Water Res. 41 (2007) 1697–1704.
[6] P. Horton, A.V. Ruban, R.G. Walters, Regulation of light harvesting in green plants, Plant Physiol. 106 (1994) 415–420.
[7] J.E. Toro, The growth rate of two species of microalgae used in shellfish hatcheries cultured under two light regimes, Aqua. Fish. Manage. 20 (1989) 249–254.
[8] L. Sicko-Goad, N.A. Andresen, Effect of growth and light/dark cycles on diatom lipid content and composition, J. Phycol. 27 (1991) 710–718.
[9] O. Pulz, K. Scheinbenbogen, Photobioreactors: design and performance with respect to light energy input, Adv. Biochem. Eng. Biotechnol. 59 (1998) 123–152.
[10] E. Molina Grima, F.G.A. Fernandéz, F.G. Camacho, Y. Chisti, Photobioreactors: light regime, mass transfer, and scale up, J. Biotechnol. 70 (1999) 231–247.
[11] Y. Kitaya, H. Azuma, M. Kiyota, Effects of temperature, CO_2/O_2 concentrations and light intensity on cellular multiplication of microalgae *Euglena gracilis*, Adv. Space Res. 35 (2005) 1584–1588.
[12] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic assignments strain histories and properties of pure cultures of cyanobacteria, J. Gen. Microbiol. 111 (1979) 1–61.
[13] E. Jacob-Lopes, L.M.C.F. Lacerda, T.T. Franco, Biomass production and carbon dioxide fixation by Aphanothece microscopica Nägeli in a bubble column photobioreactor, Biochem. Eng. J. 40 (2008) 27–34.
[14] M.I. Queiroz, E. Jacob-Lopes, L.Q. Zepka, R. Bastos, R. Goldbeck, The kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor, Bioresour. Technol. 98 (2007) 2163–2169.
[15] M. Janssen, T.C. Kuijpers, B. Veldhoen, M.B. Ternbach, J. Tramper, L.R. Mur, R.H. Wijffels, Specific growth rate of *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* under medium duration light:dark cycles: 13–87 s, J. Biotechnol. 70 (1999) 323–333.
[16] M. Janssen, M. Winter, J. Tramper, L.R. Mur, J. Snel, R.H. Wijffels, Efficiency of light utilization of *Chlamydomonas reinhardtii* under medium-duration light:dark cycles, J. Biotechnol. 78 (2000) 123–137.
[17] M. Janssen, P. Slenders, M. Winter, J. Tramper, L.R. Mur, R.H. Wijffels, Photosynthetic efficiency of *Dunaliella tertiolecta* under short light/dark cycles, Enzyme Microb. Technol. 29 (2001) 298–305.
[18] E.G. Evers, A model for light-limited continuous cultures—growth, shading and maintenance, Biotechnol. Bioeng. 38 (1991) 254–259.
[19] J.U. Grobbelaar, L. Nedbal, V. Tichy, Influence of high frequency light:dark fluctuations on photosynthetic characteristics of microalgae photoacclimated to different light intensities and implications for mass algal cultivation, J. Appl. Phycol. 8 (1996) 335–343.
[20] P. Fay, The Blue Greens (Cyanophyta: Cyanobacteria), 5 ed., Edward Arnold, London, 1983, p. 88.
[21] E. Zak, B. Norling, R. Maintra, F. Huang, B. Andersson, B. Pakrasi, The initial steps of biogenesis of cyanobacterial photosystems occurs in plasma membranes, Plant Biol. 32 (2001) 13443–13448.
[22] F.R. Martins, E.B. Pereira, S.L. Abreu, Satellite-derived solar resource maps for Brazil under SWERA project, Sol. Energy 81 (2007) 517–528.