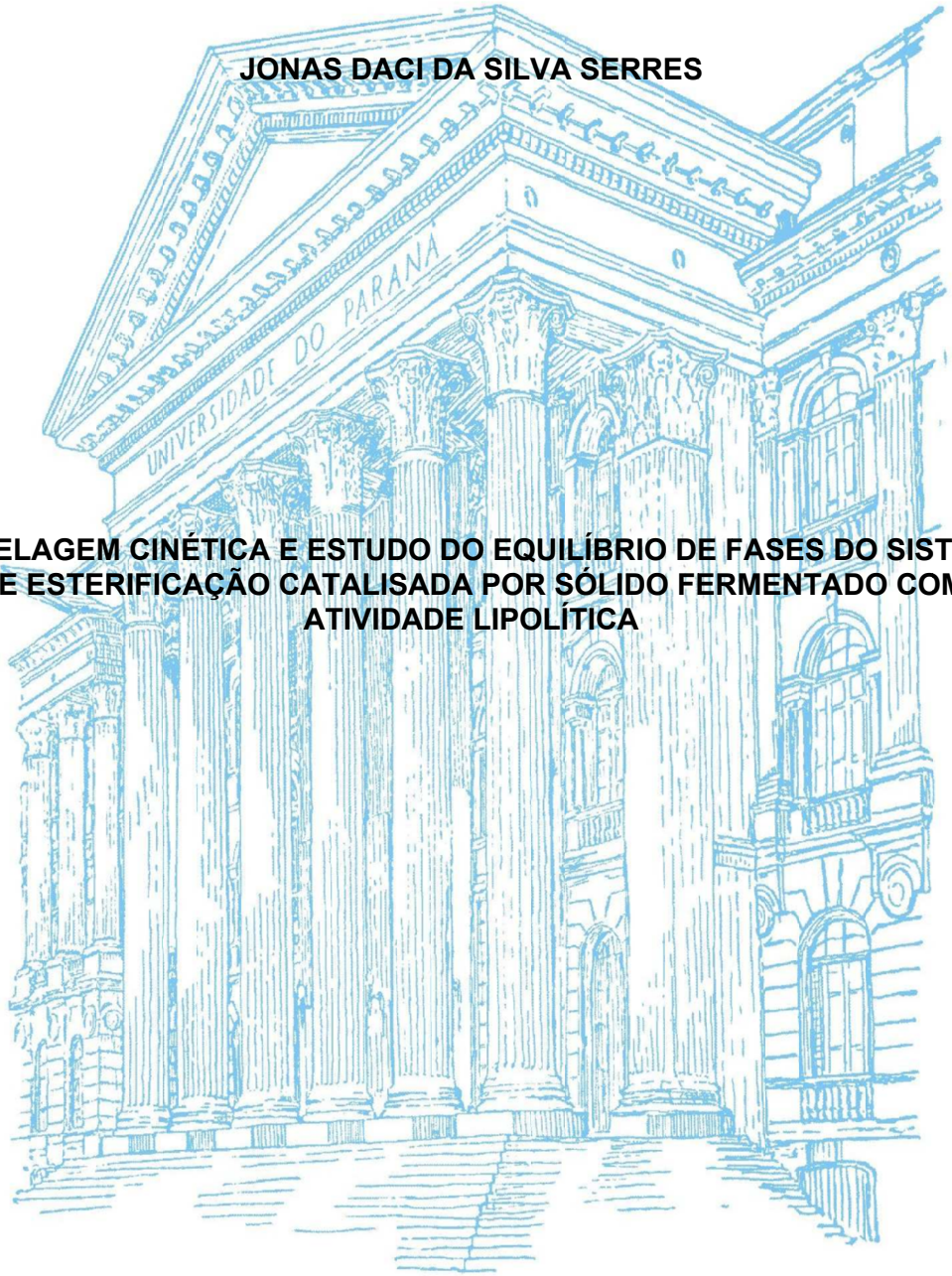
UNIVERSIDADE FEDERAL DO PARANÁ

JONAS DACI DA SILVA SERRES



MODELAGEM CINÉTICA E ESTUDO DO EQUILÍBRIO DE FASES DO SISTEMA DE ESTERIFICAÇÃO CATALISADA POR SÓLIDO FERMENTADO COM ATIVIDADE LIPOLÍTICA

CURITIBA
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Tese apresentada ao Programa de Pós-Graduação em Ciências: Bioquímica, Setor de Ciências Biológicas da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Bioquímica.

Orientador: David Alexander Mitchell

Coorientadores: Marcos Lúcio Corazza e Nadia Krieger

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TERMO DE APROVAÇÃO

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Dedico este trabalho à minha mãe, Maria Amélia da Silva Serres.

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“Pouco conhecimento faz que as criaturas sintam-se orgulhosas.”

Leonardo da Vinci

RESUMO

Na última década, o interesse pela produção de biodiesel catalisada por lipases tem aumentado consideravelmente. Uma das principais razões para isto deve-se à catálise enzimática representar uma alternativa “verde” à catálise química tradicionalmente empregada na indústria. Entretanto, embora a rota enzimática forneça produto de maior qualidade, em condições de reação mais amenas e ausência de reações laterais, sua implantação em escala industrial ainda é limitada pelos altos custos do biocatalisador. Recentemente, estratégias visando reduzir os custos de produção de biocatalisadores têm sido estudadas. Uma dessas estratégias é a produção de lipases por fermentação em estado sólido e a consecutiva aplicação destes sólidos fermentados diretamente ao meio reacional para catalisar reações de transesterificação ou esterificação. Outra estratégia para reduzir os custos na produção enzimática de biodiesel é realizar reações em sistemas livres de solventes, o que permite o uso de reatores menores e a redução do impacto ambiental e das etapas de extração e recuperação do biodiesel. Além disso, a utilização de matérias-primas residuais, como as borras ácidas, para a produção enzimática de biodiesel oferece possibilidade de reduzir significativamente o custo do processo, uma vez que a matéria-prima representa cerca de 70-85% do custo total de produção. O uso de sólido fermentado para a produção de ésteres do biodiesel a partir de ácidos graxos da borra do óleo de soja em sistema livre de solvente foi investigado recentemente, onde foi mostrado que cerca de 30% do meio reacional era sorvido pelo sólido fermentado e que este meio sorvido tinha composição diferente da encontrada para o meio de reação líquido. A composição dessa fase sorvida, que representa o microambiente da lipase no sólido, tem implicações diretas na modelagem cinética da reação. Assim, o objetivo deste trabalho foi desenvolver um modelo cinético combinado com o efeito de sorção para descrever a reação de esterificação de ácidos graxos da borra ácida do óleo de soja catalisada por sólido fermentado com atividade lipolítica em sistema livre de solvente. A sorção dos componentes do meio ao sólido fermentado seguiu uma isoterma de Langmuir multicomponente, enquanto a equação cinética foi expressa em termos de composição da fase sorvida. Com um único conjunto de parâmetros, foi possível encontrar bom ajuste do modelo aos dados experimentais obtidos nos estudos anteriores para três razões molares etanol:ácidos graxos (1:1, 1,5:1, 3:1), tanto para perfis de conversão quanto para composição nas fases líquida e sorvida. Além disso, foram construídos diagramas ternários experimentais de Equilíbrio Líquido-Líquido para o sistema “ácidos graxos + etanol + água + ésteres etílicos”, e também foi feita a modelagem termodinâmica para este sistema. Este modelo termodinâmico pode ser usado, futuramente, para prever a separação das fases orgânica e aquosa no reservatório do sistema, permitindo o uso do modelo cinético-sorção para um sistema onde somente a fase orgânica do reservatório seja retornada ao leito fixo que contém o sólido fermentado. Com isso, o modelo poderá ser utilizado para realizar o escalonamento do processo de produção enzimática de biodiesel.

Palavras-chave: biodiesel, *Burkholderia cepacia*, equilíbrio líquido-líquido, esterificação, lipase, sólido fermentado, modelagem cinética, modelagem termodinâmica

ABSTRACT

Over last decade, interest on biodiesel production catalyzed by lipases has been increased. One of the most important reasons for this is because enzymatic catalysis represents a greener alternative to chemical catalysis used on industry traditionally. However, although enzymatic route yields higher quality product at mild conditions with no side reactions its implementation in large scale is limited yet by high biocatalyst costs. Recently, strategies aiming to reduce production costs of biocatalysts have been studied. One of these strategies is producing lipases by solid state fermentation and consecutively applying these fermented solids in reaction media to catalyze transesterification and esterification reactions. Other strategy to reduce enzymatic biodiesel production costs is performing reactions in solvent-free systems, which allows using smaller reactors and decreasing environmental impact and extraction and recovery steps. Furthermore, the use of cheaper feedstocks, as soapstocks, to enzymatic production of biodiesel offers possibility of reducing process cost significantly, since feedstocks represent around 70-85% of whole production cost. Using fermented solid to produce biodiesel esters from fatty acids from soybean soapstock acid oil has been investigated recently, and it was shown that up to 30% of the total mass of the reaction medium sorbed onto the fermented solids during the process and that this sorbed phase had a composition that was quite different from that of the bulk reaction medium. Composition of sorbed phase, which represents lipase microenvironment into fermented solid, has implications in modeling reaction kinetics. Therefore, the aim of this study was develop a combined sorption-kinetic model capable of describing the reaction profiles in the esterification of fatty acids from soybean soapstock acid oil catalyzed by fermented solid with lipolytic activity in a solvent-free system. The sorption of the medium components onto the fermented solids followed a multicomponent Langmuir isotherm, while the kinetic equation was expressed in terms of the composition of the sorbed phase. With a single set of parameters, the model adjusted well to the experimental results for three different ethanol to fatty acid molar ratios (1:1, 1.5:1 and 3:1), not only for the overall conversion profile, but also for the compositions of the bulk reaction medium and the sorbed phase.

Moreover, experimental ternary diagrams of Liquid-Liquid Equilibrium of system “fatty acids + ethanol + water + ethyl esters” were constructed and also thermodynamic modeling was performed to this system. These data could be used further to predict separation of organic and aqueous phases in reaction reservoir, allowing the use of kinetic-sortion model to a system where only organic phase be recycled to the packed bed that contains fermented solid. From this perspective, model would be applied to guide scale-up of enzymatic biodiesel production.

Keywords: biodiesel, *Burkholderia cepacia*, liquid-liquid equilibrium, esterification, lipase, fermented solid, kinetic modeling, thermodynamic modeling

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LISTA DE ABREVIATURAS

AG	Ácido graxo
AGL	Ácido graxo livre
EE-SSAO	*Ethyl esters from soybean soapstock acid oil*
ELL	Equilíbrio líquido-líquido
FA-SSAO	*Fatty acids from soybean soapstock acid oil*
FES	Fermentação em estado sólido
LLE	*Liquid-liquid equilibrium*
LTEB	Laboratório de Tecnologia Enzimática e Biocatálise
ME	Metil ésteres
M_{NaOH}	*Molarity of NaOH solution*
MR	*Molar ratio*
NRTL	*Non random two liquid*
Rmsd	*Root mean square deviation*
SFBC	Sólido fermentado de *Burkholderia cepacia*
SLS	Sistema livre de solvente
SSAO	*Soybean soapstock acid oil*
SW	*sample weight*
TABEK	*Thermodynamic activity-based enzyme kinetics*
UNIFAC	*Universal Function Activity Coefficient*
UNIQUAC	*Universal QuasiChemical*

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1. INTRODUÇÃO

1.1. PRODUÇÃO ENZIMÁTICA DE BIODIESEL

Uma das reações de esterificação mais estudadas nos últimos anos é a produção de ésteres do biodiesel catalisada por lipases. Uma das principais razões disso é devido à catálise enzimática minimizar os problemas associados com a catálise química tradicional para a produção deste biocombustível (GULDHE *et al.*, 2015). A catálise enzimática inclui vantagens, tais como: processos de produção mais simples, menores requerimentos energéticos, maior pureza de subprodutos, inexistência de reação de saponificação, separação mais fácil, reutilização do catalisador e menor quantidade de água desperdiçada por processos de lavagem. Ainda, lipases podem gerar ésteres alquílicos não apenas por matérias-primas convencionais, como óleos vegetais de alta qualidade, mas também por matérias-primas residuais, como a borra ácida, que possui maiores teores de ácidos graxos livres (AGL), reduzindo, assim, os custos do processo (SOARES, 2014).

Embora a catálise enzimática exiba menores velocidades de reação, maiores custos e eventual perda de atividade do biocatalisador, a rota enzimática para a produção de biodiesel é atualmente considerada como uma alternativa mais “verde”, devido à possibilidade de reutilização do biocatalisador por técnicas de imobilização e desenvolvimento de processos mais baratos para a produção de enzimas (GULDHE *et al.*, 2015, LI e ZONG, 2010). Uma dessas técnicas é o processo de fermentação em estado sólido (FES), cujos principais resíduos que podem ser utilizados como suportes para a produção de lipases são as tortas originadas dos processos de extração do óleo de sementes de milho, girassol e soja (BHARGAV *et al.*, 2008).

Outra forma de reduzir os custos da produção enzimática de biodiesel é o uso de sistemas livres de solvente (SLS), o que minimiza o tamanho do reator e, dessa forma, seu custo, além de facilitar os processos de separação e purificação dos produtos. O uso de SLS é atrativo também por minimizar os problemas causados pelos solventes, como risco de incêndio e danos ambientais.

Com relação à utilização direta de sólidos fermentados com atividade lipolítica para produção de biodiesel, alguns estudos têm sido relatados na literatura (ZAGO *et al.*, 2014, LIU *et al.*, 2013, SALUM *et al.*, 2010, FERNANDES *et al.*, 2007) e são apresentados na Tabela 1.

Fernandes *et al.* (2007) e Salum *et al*. (2010) utilizaram, pioneiramente, em reações de esterificação e transesterificação, um sólido fermentado de *Burkholderia cepacia* LTEB11 (SFBC) liofilizado, produzido no Laboratório de Tecnologia Enzimática e Biocatálise (LTEB) da UFPR. Nos estudos de esterificação e transesterificação, Fernandes *et al*. (2007) obtiveram 94 e 95% de conversões em ésteres em 18 e 120 h, respectivamente. Posteriormente, Salum *et al*. (2010) otimizaram a FES e produziram um novo SFBC com o dobro da atividade obtida por Fernandes *et al*. (2007). Este sólido foi, então, utilizado em reações de transesterificação em sistemas livres de solvente com conversões em ésteres de 95% em 46 h de reação.

Soares *et al*. (2013) investigaram a síntese de ésteres do biodiesel a partir de material de partida residual, a borra ácida de óleo de soja, em SLS em um biorreator de leito fixo contendo sólido fermentado com atividade lipolítica (SOARES *et al.*, 2013). A conversão em ésteres obtida foi de 92% em 31 h de reação. Já Liu *et al*. (2013) empregaram um sólido fermentado de *Burkholderia cenocepacia* em reações de transesterificação do óleo de soja para produção de biodiesel (LIU *et al.*, 2013) com conversão em ésteres de 86% em 96 h.

Recentemente, Zago *et al*. (2014) produziram um sólido fermentado de *Rhizopus microsporus* e o aplicaram para a síntese de ésteres etílicos a partir de óleo de milho (ZAGO *et al.*, 2014), obtendo 91% de conversão em ésteres em 48 h de reação. Aguieiras *et al*. (2014) investigaram a esterificação enzimática a partir do óleo ácido da polpa de macaúba (*Acrocomia aculeata*), um material de partida de baixo custo, para a produção de biodiesel em SLS com conversão em ésteres de 91% em 8 h. O teor e a qualidade dos ésteres obtidos ao final do processo estavam de acordo com as normas brasileiras para o biodiesel (AGUIEIRAS *et al.*, 2014).

TABELA 1 - ESTUDOS QUE UTILIZARAM SÓLIDOS FERMENTADOS PARA SÍNTESE DE ÉSTERES DO BIODIESEL

Micro-organismo	Reação	Suporte	Matéria-prima	Álcool	Razão molar (álcool/óleo)	Solvente	Conversão (%)	Tempo (h)	Reator (modo)	Referência
Burkholderia cepacia	Esterificação/ Transesterificação	Bagaço de cana-de-açúcar e farelo de semente de girassol	Ácido oleico/óleo de milho	Etanol	5:1/6:1	*n*-heptano	94/95	18/120	Frasco (batelada)	Fernandes *et al*. (2007)
Burkholderia cepacia	Transesterificação	Bagaço de cana-de-açúcar e farelo de semente de girassol	Óleo de soja	Etanol	3:1	Ausência	95	46	Leito fixo (batelada)	Salum *et al*. (2010)
Burkholderia cepacia	Esterificação	Bagaço de cana-de-açúcar e farelo de semente de girassol	Ácidos graxos da borra ácida do óleo de soja	Etanol	3:1	Ausência	92	31	Leito fixo (batelada)	Soares *et al*. (2013)
Burkholderia cenocepacia	Transesterificação	Bagaço de cana-de-açúcar e farelo de semente de girassol	Óleo de soja	Etanol	4:1	*t*-butanol	86	96	Frasco (batelada)	Liu *et al*. (2013) Liu *et al*. (2014)
Rhizopus microsporus	Transesterificação	Bagaço de cana-de-açúcar e farelo de semente de girassol	Óleo de milho	Etanol	3:1	*n*-heptano	91	48	Frasco (batelada)	Zago *et al*. (2014)
Rhizomucor miehei	Esterificação	Torta de babassu	Óleo ácido da polpa de macauba	Etanol	2:1	Ausência	91	8	Frasco (batelada)	Aguieiras *et al*. (2014)

Os resultados relatados indicam o potencial da aplicação direta de sólidos fermentados com atividade lipolítica na síntese de ésteres, principalmente ésteres etílicos de ácidos graxos de cadeia longa, componentes do biodiesel. Vale ressaltar que a adição direta do sólido fermentado ao meio reacional evita a necessidade de etapas adicionais de extração e imobilização da enzima e, portanto, tem potencial para reduzir o custo da síntese de biodiesel catalisada por lipases.

Apesar de estes trabalhos mostrarem o uso de sólido fermentado para síntese de ésteres do biodiesel, nenhum deles, além de Liu *et al*. (2014) realizou estudos cinéticos do sistema reacional, o que seria de grande interesse para o desenvolvimento e otimização de processos biocatalíticos em larga escala.

1.2. CINÉTICA E TERMODINÂMICA DE MEIOS MULTIFÁSICOS

Apesar das vantagens relacionadas ao uso de SLS para reações de esterificação, esses sistemas geralmente formam duas fases ou mais e o seu controle difere consideravelmente quando comparado a sistemas monofásicos convencionais. Um dos fatores para avaliação da reação em SLS é o estado de equilíbrio, cujo indicador numérico é uma constante de equilíbrio, que se trata de um parâmetro intrínseco e definida pelas atividades dos reagentes, ou seja, substratos e produtos. Por isso, seu valor depende apenas da temperatura e não de outros fatores, como tipo de solvente, por exemplo. No entanto, há poucos estudos tratando da análise do estado de equilíbrio em SLS para produção de biodiesel (KOBAYASHI *et al.*, 2007, DOSSAT *et al.*, 2002, TEWARI e BUNK, 2001) e a previsão do comportamento da reação é difícil.

Ainda, a presença do biocatalisador imobilizado em um suporte sólido gera uma terceira fase (FIGURA 1), onde as limitações de transferência de massa podem ser significativas na determinação da cinética de reação. Nessas reações de esterificação em sistemas multifásicos, o equilíbrio encontra-se entre hidrólise e síntese, dependendo altamente do teor de água no meio reacional, conforme a hidrólise e a esterificação são promovidas por sistemas macro- e microaquosos, respectivamente. Assim, a fim de descrever o sistema sob consideração, suposições adequadas precisam ser feitas nos passos de reação elementares, ou seja, para

sugerir um mecanismo e estimar valores de parâmetros cinéticos usando dados experimentais e equações apropriadas (STERGIOU *et al.*, 2013).

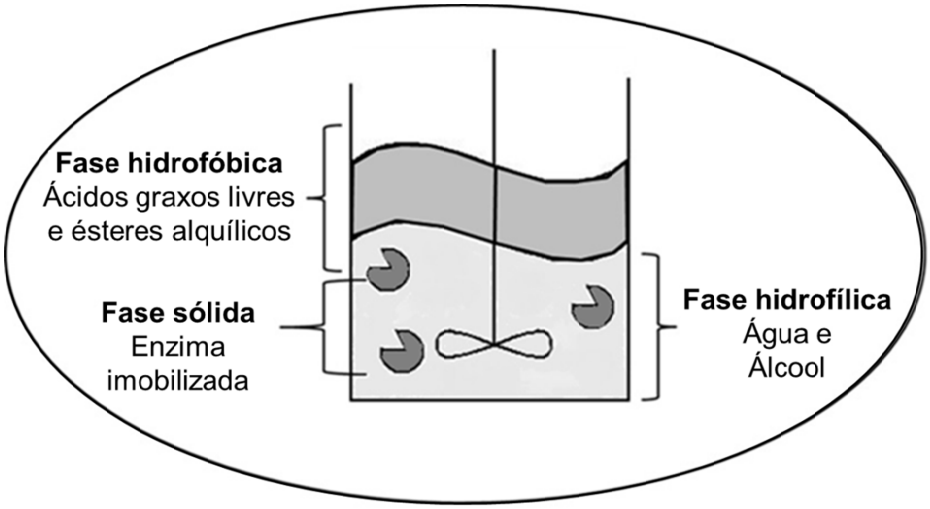


FIGURA 1 - ILUSTRAÇÃO DAS TRÊS FASES DO SISTEMA REACIONAL EMPREGADO PARA UMA REAÇÃO DE ESTERIFICAÇÃO CATALISADA POR ENZIMA IMOBILIZADA EM SISTEMA LIVRE DE SOLVENTE
FONTE - ADAPTADO DE XU (2012)

1.2.1. Modelagem da cinética de reação em sistemas multifásicos

A cinética de reações de esterificação catalisadas por lipases em SLS tem sido estudada nos últimos anos (MAHMUD *et al.*, 2010, FORESTI *et al.*, 2008, SANDOVAL *et al.*, 2002, GARCIA *et al.*, 2000, GARCIA *et al.*, 1999). Um dos primeiros trabalhos a abordar este tema foi o de Sandoval *et al*. (2002), que descreveram a cinética de reação de esterificação de ácido oleico com etanol em SLS catalisada pela lipase comercial (Lipozyme®) de *Rhizomucor miehei* imobilizada em resina aniônica macroporosa (Duolite A568)(SANDOVAL *et al.*, 2002). Eles assumiram que a polaridade do etanol interferiria na disponibilidade de reagentes e admitiram que os parâmetros cinéticos nesses sistemas eram diferentes daqueles determinados em sistemas com *n*-hexano (CHULALAKSANANUKUL *et al.*, 1990). Consequentemente, esses parâmetros seriam diferentes para cada razão molar dos substratos. Para caracterizar o sistema, eles usaram um modelo que levava em conta as diferenças na disponibilidade dos reagentes devido a mudanças no meio de reação, que eles denominaram como TABEK (do inglês *Thermodynamic Activity-Based Enzyme Kinetic*s). Nesse modelo, os parâmetros cinéticos eram

independentes da natureza do solvente e seus valores eram determinados pelas atividades termodinâmicas dos reagentes, ao invés de suas concentrações.

Para isso, eles substituíram a equação baseada em concentrações, proposta por Chulalaksananukul *et al*. (1990),

$$V_i = \frac{V_{\max}[Ac][Et]}{K_{mAc}\left(1+\frac{[Et]}{K_i}\right)[Et]+K_{mEt}[Ac]+[Ac][Et]} \quad (1)$$

por uma que fosse baseada nas atividades (a) dos reagentes,

$$V_i = \frac{V_{\max}\cdot a_{Ac}a_{Et}}{K_{mAc}\left(1+\frac{a_{Et}}{K_i}\right)a_{Et}+K_{mEt}a_{Ac}+a_{Ac}a_{Et}} \quad (2)$$

como a atividade de um componente é o produto entre um coeficiente de atividade e sua concentração, eles determinaram os coeficientes de atividade pelo método de contribuição de grupo UNIFAC.

Eles avaliaram a inibição da reação pelo álcool e descobriram que este efeito do etanol em SLS, embora exista, é bem menor do que em solventes, como *n*-hexano. Em altas razões molares de etanol para ácido oleico (por exemplo, 4:1) a polaridade global do meio reacional aumentou, o que diminuiu a disponibilidade de etanol e, consequentemente, seu efeito inibitório em SLS. Além disso, em altas razões molares iniciais, o etanol agiu como um solvente, amenizando mudanças na polaridade. Eles também observaram que o perfil de conversões era o resultado de efeitos combinados da lei de ação das massas, partição da água entre a fase líquida e o suporte da enzima e a evolução dos coeficientes de atividade dos reagentes. Eles quantificaram esses efeitos pela determinação de uma “constante de equilíbrio intrínseca”, baseada nas atividades termodinâmicas dos reagentes. Eles também consideraram a partição de água entre o meio da reação e o suporte enzimático, o que influenciaria no equilíbrio por modificar a quantidade de água dissolvida no meio reacional.

Uma das grandes limitações da abordagem de Sandoval *et al*. (2002) é que a constante de equilíbrio que eles encontraram variava significativamente com as razões molares iniciais de etanol para ácido oleico. Eles associaram isso ao fato de que o cálculo matemático de K_{eq} era muito sensível ao valor de conversão e um leve aumento na conversão experimental conduziria a um aumento significativo no valor calculado de K_{eq}.

Além disso, eles verificaram que a conversão em éster em equilíbrio dependia fortemente da quantidade de suporte adicionado ao meio reacional, o que eles relacionaram à hidrofilicidade do suporte da enzima, uma vez que diminuiria a quantidade de água dissolvida no sistema e deslocaria o equilíbrio para a reação de síntese. É interessante notar que nenhuma menção sobre a existência de um meio bifásico foi feita, o que é surpreendente, já que a análise termodinâmica prevê a existência de duas fases no sistema no decorrer da reação (BUCALÁ *et al.*, 2006).

O caráter bifásico do meio reacional para a síntese do oleato de etila catalisada por lipase imobilizada em pó de quitosana em SLS foi considerada por Bucalá *et al*. (2006) para modelar as condições de equilíbrio da reação. Neste trabalho, eles determinaram matematicamente a constante de equilíbrio químico para a esterificação de ácido oleico e uma mistura de ácidos graxos catalisada por lipase em SLS. Para isso, eles propuseram que ambos os equilíbrios de fase e de reação fossem resolvidos simultaneamente, fornecendo estimativas precisas de dados de equilíbrio experimentais.

Este estudo indicou que a correlação da constante de equilíbrio como função das condições de operação, como tinha sido feita por Sandoval *et al*. (2002), não é necessária para representar o equilíbrio químico do sistema bifásico. Conversões em equilíbrio experimentais foram bem representadas por uma constante de equilíbrio termodinâmica, dependente da temperatura e calculada a partir de um procedimento de otimização. Eles obtiveram bons resultados para uma ampla faixa de composições do sistema reacional, incluindo para sistemas com altos teores iniciais de água. Os resultados indicaram que o estado de Equilíbrio Líquido-Líquido (ELL) não pode ser desconsiderado se boas estimativas de conversões em equilíbrio são desejadas.

Em outro trabalho, Foresti *et al*. (2008) propuseram um modelo matemático para a mesma reação do trabalho de Bucalá *et al*. (2006), para determinar as composições dos reagentes durante o tempo e ajustar os parâmetros cinéticos da

reação, considerando a transferência de massa e a reação química ao mesmo tempo (FORESTI *et al.*, 2008). Neste trabalho, a equação cinética que eles escolheram é derivada do mecanismo Pingue-Pongue Bi Bi, considerando a inibição competitiva por etanol e a velocidade da reação inversa. A equação é dada por:

$$v = \frac{V_d V_i \left([Ac][Et] - \frac{[Ag][Es]}{K_{eq}} \right)}{V_i K_{mEt}[Ac] + V_i K_{mAc}[Et] + \frac{V_i K_{mEs}[Ag]}{K_{eq}} + \frac{V_d K_{mAg}[Es]}{K_{eq}} + V_i[Ac][Et] + \frac{V_d K_{mEs}[Ac][Ag]}{K_{eq} K_{iAc}} + \frac{V_d[Ag][Es]}{K_{eq}} + \frac{V_i K_{mAc}[Et][Es]}{K_{iEs}}} \quad (3)$$

onde K_{eq} é a constante de equilíbrio da reação, V_d é a velocidade de reação direta, V_i é a velocidade de reação inversa; [Ac], [Et], [Ag] e [Es] são as concentrações de ácido oleico, etanol, água e éster, respectivamente e K_{mAc}, K_{mEt}, K_{mAg}, K_{mEs}, K_{iAc} e K_{iEs} são as respectivas constantes cinéticas.

A fim de modelar a cinética da reação de esterificação em um reator do tipo tanque agitado em modo batelada, eles assumiram duas condições iniciais: nenhuma quantidade de água adicionada, o que configurou o sistema como monofásico no início da reação; e com quantidades diferentes de água adicionadas ao início da reação, 10 e 20% (m/m), configurando um sistema bifásico. Entre as suposições adotadas para o modelo, estavam: reação isotérmica, desconsideração da fase sólida do suporte da enzima, limitações de transferência de massa interna e externa negligenciáveis, fase orgânica como fase reacional, distribuição homogênea do catalisador entre as duas fases.

O modelo bifásico apresentado foi comparado com a abordagem monofásica tradicional, que desconsiderava a formação de duas fases ao longo da reação e que não foi capaz de representar os dados experimentais corretamente, enquanto o modelo bifásico permitiu um bom ajuste aos dados. Entretanto, com a suposição de distribuição homogênea do catalisador (lipase imobilizada em pó de quitosana) entre as duas fases, eles assumiram que a razão de massa do catalisador nas duas fases foi igual à razão de volumes das duas fases. Eles não mencionaram a possibilidade de existir uma fase específica com uma composição particular sorvida nas partículas sólidas do material de suporte. Eles assumiram que a reação ocorreu apenas na

fase orgânica, sem determinar as composições das duas fases experimentalmente, confiando apenas na modelagem termodinâmica para prever as composições das fases. Isso poderia ser razoável para o sistema que eles estudaram, formado por componentes puros, mas não seria para sistemas onde há uma matéria-prima complexa, como a borra ácida, por exemplo, que foi usada por Soares *et al*. (2015).

Em um estudo semelhante, Krause e Fieg (2011) desenvolveram um modelo cinético para a hidrólise de uma mistura de ésteres metílicos de cadeia curta em um sistema bifásico orgânico/aquoso, com esta reação sendo catalisada pela lipase de *Candida antarctica* B Novozym 435 (uma lipase comercial imobilizada em poli(meta-acrilato) (KRAUSE e FIEG, 2011). Para a modelagem da reação de hidrólise em reator de leito fixo, eles tiveram de considerar as duas fases líquidas combinadas com o equilíbrio de fase e de reação, bem como a reação cinética. Além disso, um comportamento termodinâmico de sistema não ideal foi assumido, o que os levou a quantificar as não-idealidades do sistema pelo uso de atividades termodinâmicas nas relações cinéticas e de equilíbrio, uma abordagem parecida com a que Sandoval *et al*. (2002) executaram e como já fora descrito anteriormente.

A fim de investigar a influência da quantidade de água dentro da partícula do catalisador na velocidade de reação, eles examinaram os escoamentos das duas fases dentro do reator qualitativamente, através de microscopia óptica. Eles observaram que as partículas do biocatalisador Novozym 435 exibiam um caráter hidrofóbico e que um filme de fase orgânica cobria a superfície das partículas e impedia o contato da enzima com a fase aquosa. Eles também observaram que, em baixa fração de fase orgânica no sistema, havia formação de canais de fluxo inter-partículas pela fase orgânica que envolvia as partículas do catalisador. A fase orgânica escoava através do leito pela superfície das partículas e por esses canais. A fase aquosa nesse sistema era contínua e escoava de maneira pulsante e não entrava em contato com as partículas do catalisador enquanto estas estavam preenchidas com fase orgânica. Em altas velocidades de fluxo, entretanto, eles observaram que a fase orgânica continha uma fina dispersão de gotículas da fase aquosa.

A partir dos resultados obtidos, eles consideraram que a reação de hidrólise acontecia na fase orgânica que envolvia as partículas da enzima e tanto a cinética da reação quanto o equilíbrio químico foram formulados para esta fase, sendo que a fase aquosa foi incorporada apenas nos cálculos de ELL. Partindo da mesma

abordagem proposta por Bucalá *et al*. (2006), eles usaram atividades termodinâmicas para determinar as constantes de equilíbrio das reações de hidrólise, que são definidas como

$$K_{eq,n} = \frac{a_{AGn,org} a_{MeOH,org}}{a_{MEn,org} a_{Ag,org}} \tag{4}$$

onde *n* define o número de carbonos na cadeia carbônica de ácidos graxos (AG) e ésteres metílicos (ME): AGn=C[4+2n]AG, MEn=C[4+2n]ME.

Através do mecanismo proposto por Malcatá *et al*. (1992), um modelo cinético multirresposta para a hidrólise de óleo de manteiga catalisada por lipase imobilizada (MALCATA *et al.*, 1992), Krause e Fieg (2011) obtiveram a expressão cinética para hidrólise de ésteres dada por:

$$r_{éster,i} = \frac{\Theta_{1,i}[Es]_i[H_2O] - \Theta_{2,i}[AG]_i[MeOH]}{1 + \sum_{i=1}^{N} \Theta_{3,i}[Es]_i} \tag{5}$$

onde Θ, com subscritos apropriados, representa parâmetros cinéticos agrupados.

A fim de obter consistência entre as constantes de equilíbrio e a abordagem cinética, eles substituíram concentrações por atividades termodinâmicas na expressão cinética, o que melhorou a descrição dos resultados experimentais e simplificou a combinação matemática do termo de equilíbrio e cinética. A equação resultante é representada por:

$$r_{MEn,org} = \frac{1}{m_{cat}} \frac{dn_{MEn,org}}{dt} = \frac{k_{1,n}\left(a_{MEn,org} a_{H_2O,org} - \left(\frac{1}{K_{eq,n}} \right) a_{AGn,org} a_{MeOH,org} \right)}{1 + \sum_{n=1}^{3} \left(k_{2,n} a_{MEn,org} \right)} \tag{6}$$

Com base na suposição de que o reator se aproximava do comportamento de tanque agitado, eles determinaram os parâmetros cinéticos da hidrólise para três

ésteres metílicos (hexanoato de metila, octanoato de metila e decanoato de metila). O modelo ajustou-se bem aos dados experimentais. Apesar das importantes contribuições deste trabalho no campo da modelagem cinética de reações catalisadas por lipases em sistemas bifásicos, vale ressaltar que tal abordagem não seria apropriada para um sistema onde o catalisador seja um sólido fermentado com atividade lipolítica. Nesse caso, considerações sobre a influência do material de suporte da enzima na reação precisariam ser realizadas, o que é o interesse do presente trabalho.

Com relação à modelagem de reações catalisadas por sólidos fermentados com atividade lipolítica, há apenas o trabalho de Liu *et al*. (2014). Nesse estudo, eles investigaram a cinética e a otimização da transesterificação de óleo de soja com etanol catalisada por sólido fermentado com atividade lipolítica, em um sistema com *terc*-butanol como solvente (LIU *et al.*, 2014). Para isso, eles propuseram um modelo cinético para etanólise baseado no mecanismo Pingue-Pongue Bi Bi, com inibição competitiva por etanol. Sua equação cinética é dada por:

$$v = \frac{v_{\max}[TG][Et]}{K_{mTG}[Et]\left(1+\frac{[Et]}{K_i}\right)+K_{mEt}[TG]+[TG][Et]} \tag{7}$$

onde [TG] e [Et] são as concentrações molares de triglicerídeo e etanol, respectivamente; K_{mTG} e K_{mEt} são as constantes de Michaelis-Menten do TG e Et e K_i é a constante de inibição do etanol.

A partir de uma comparação da Equação (7) com a Equação (3), pode-se perceber que o termo da reação inversa foi ignorado no numerador, o que implica supor que a reação é irreversível e que procederá até que o reagente limitante seja totalmente consumido, o que não acontece em sistemas de esterificação relacionados à produção de biodiesel. Também vale ressaltar que nenhuma menção foi feita sobre a influência do material sólido na velocidade de reação, já que se trata do mesmo material usado por Soares *et al*. (2015), uma mistura 1:1 (m/m) de bagaço de cana-de-açúcar e farelo de semente de girassol (SOARES *et al.*, 2015). Como afirmado por Soares *et al*. (2015), o suporte sólido empregado na FES interage com os componentes da reação (etanol, ácidos graxos do óleo ácido da

borra de soja, ésteres etílicos destes ácidos graxos e água) de uma maneira complexa. Apesar de o sólido ser constituído por mistura 1:1 de bagaço de cana, com caráter hidrofílico, e farelo de semente de girassol, com caráter hidrofóbico, o suporte sorve maior quantidade de componentes hidrofílicos (água e etanol), aumentando drasticamente a razão molar de etanol para ácidos graxos na fase sorvida (em torno de 1762:1 quando comparado à razão molar inicial de 3:1 na fase líquida). Isso claramente demonstra a importância de considerar a influência da sorção dos componentes reacionais no suporte sólido quando se aplica sólidos fermentados para catalisar reações, principalmente no que se refere à caracterização da cinética de reação. Além disso, o estudo do comportamento de fases dos sistemas reacionais multifásicos é também necessário, uma vez que fornecerá informações sobre como o sistema se comportará no decorrer da reação.

1.2.2. Análise termodinâmica de sistemas multifásicos

O comportamento de biocatalisadores em meios multifásicos é mais difícil de prever do que em sistemas monofásicos. Isso é devido à falta de experiência com esses sistemas e à sua maior complexidade. Entretanto, algumas previsões úteis podem ser realizadas através de análises termodinâmicas. Reações de esterificação em SLS catalisadas por lipases apresentam, pelo menos, duas fases distintas. Uma fase, usualmente a mais volumosa, age como um reservatório para os reagentes e produtos (ou parte deles), mas geralmente não contém o biocatalisador. Esta fase é frequentemente uma mistura líquida orgânica formada pelos reagentes. A outra fase contém o biocatalisador e pode ser uma solução aquosa diluída ou uma microfase líquida aquosa envolvendo a enzima imobilizada. Entre essas fases, reside uma ou mais interfaces, cujas propriedades podem ser muito importantes no comportamento cinético observado. Entretanto, na análise termodinâmica de equilíbrio, as interfaces podem ser negligenciadas, uma vez que somente uma pequena porção de moléculas presentes é afetada por uma interface em qualquer instante. Assim, interfaces não contribuem significativamente para a posição de equilíbrio na maioria dos sistemas (HALLING, 1994).

Frequentemente, há interesse nos componentes que se distribuem em ambas as fases, a não aquosa e a aquosa. Tais componentes incluem os substratos (ácidos

graxos e álcool) e os produtos (água e ésteres). Dessa forma, considerações termodinâmicas podem ser aplicadas a sistemas reacionais multifásicos para biocatálise, apesar de muitos desses sistemas não estarem em um estado de equilíbrio (o que é necessário para a aplicação precisa da termodinâmica-padrão). Por outro lado, muitos desses processos aproximam-se deste estado através do equilíbrio de fases líquido-líquido e a análise termodinâmica pode fornecer informações sobre esses sistemas. Além disso, essa análise pode indicar a direção das mudanças contínuas que ocorrem nesses meios reacionais.

Um importante conceito na termodinâmica é o estado de equilíbrio de um sistema, cujas características gerais são:

- Esse estado não varia com o tempo;
- O sistema é uniforme ou é formado por subsistemas uniformes, sem gradientes de temperatura, pressão, velocidade ou concentração;
- Os fluxos resultantes de massa, calor ou trabalho entre o sistema e suas vizinhanças são nulos;
- A velocidade resultante de todas as reações químicas é nula (SANDLER, 2000).

Em sistemas isotérmicos e isobáricos, o critério de equilíbrio é a variação nula de energia livre de Gibbs (ΔG) e o de estabilidade é $d^2G > 0$ (SANDLER, 2000).

Os critérios de estabilidade podem ser aplicados também para sistemas multicomponentes com a presença de mais de uma fase, resultando nos critérios para o equilíbrio de fases mostrado pelas Equações (8)-(10), que considera a existência de duas fases no sistema (SANDLER, 2000), indicadas pelos sobrescritos I e II:

$$T^I = T^{II} \tag{8}$$

$$P^I = P^{II} \tag{9}$$

$$\mu_i^I = \mu_i^{II} \tag{10}$$

Uma mistura líquida composta por diferentes espécies químicas em algumas faixas de composição pode não satisfazer o critério de estabilidade $d^2G > 0$, formando, então, duas fases líquidas com composições distintas. Quando estas fases estão em equilíbrio termodinâmico, diz-se que o sistema está em Equilíbrio

Líquido-Líquido (ELL). Determina-se a quantidade de um componente em determinada fase através de estudos de ELL e os dados obtidos são representados por diagramas de sistemas binários, ternários ou quaternários (quando o sistema possui dois, três ou quatro componentes, respectivamente).

O diagrama para sistemas ternários apresenta a forma de um triângulo equilátero e cada ponto localizado nas bordas ou dentro deste triângulo representa uma composição de mistura (FIGURA 2). A Figura 2 representa o tipo mais comum de sistemas ternários, onde dois componentes são imiscíveis entre si (componentes 1 e 3) e um componente é miscível em ambos (componente 2), como, por exemplo, o sistema inicialmente presente em reações de esterificação água + etanol + ácidos graxos.

A curva binodal, que aparece como uma linha contínua no diagrama ternário mostrado na Figura 2, mostra os limites de miscibilidade. Na região abaixo dessa curva, como, por exemplo, um sistema com a composição global representada pelo próprio ponto M, o sistema apresenta duas fases, ou seja, é um sistema heterogêneo. Acima dessa região, qualquer ponto representa um sistema com apenas uma fase (sistema homogêneo).

Finalmente, o diagrama apresenta as linhas de amarração (ou *tie-lines*), que são as linhas tracejadas da Figura 2. Estas linhas de amarração servem para conectar os pontos que representam as composições das duas fases que estão em ELL. Por exemplo, caso uma mistura com a composição do ponto M fosse deixada em repouso, ela separaria em duas fases: uma fase com a composição do ponto E, e a outra fase com a composição do ponto R. Vale ressaltar ainda que a inclinação das linhas de amarração indica a preferência do componente miscível em ambas as fases por uma fase em específico (neste caso, o componente 2 é o componente que se distribui em maior quantidade na fase rica no componente 3).

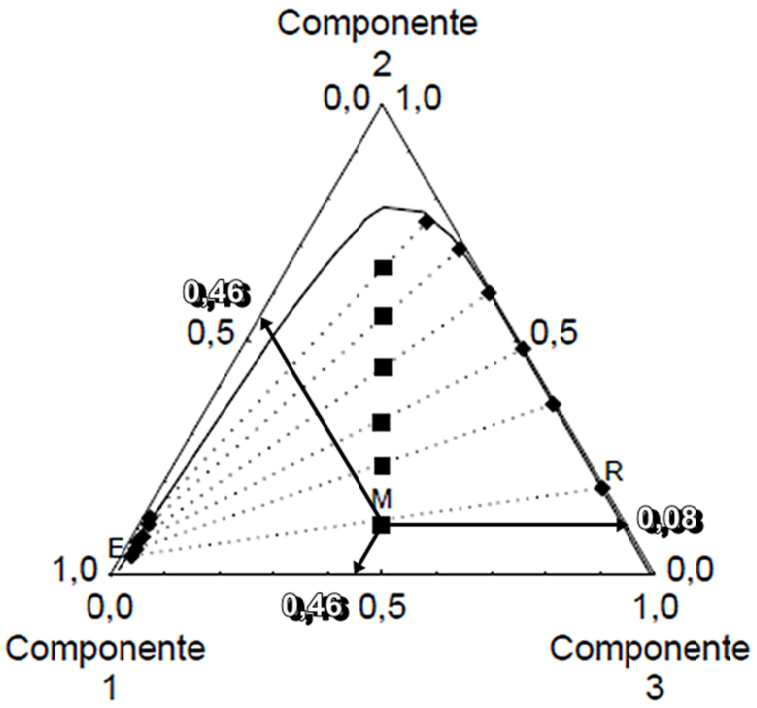


FIGURA 2 - DIAGRAMA DE FASES PARA REPRESENTAÇÃO DAS COMPOSIÇÕES DE UM SISTEMA TERNÁRIO
Cada vértice deste triângulo representa um componente puro e cada lado representa uma mistura binária. A leitura da composição do ponto M, por exemplo, é feita traçando-se retas paralelas a cada um dos lados do triângulo, sendo que cada reta é oposta ao vértice de composição máxima do componente que se deseja determinar. No caso do ponto M, a composição do componente 1 é 0,46, do componente 2 é 0,08 e do componente 3 é 0,46 (em fração mássica ou molar).
FONTE - ADAPTADO DE KANDA, (2013).

Para sistemas quaternários a representação é feita tridimensionalmente, a partir dos quatro diagramas ternários obtidos, por exemplo, um sistema composto pelos componentes A, B, C, D, geraria 4 triângulos: ABC, ABD, ACD e BCD. A união tridimensional desses triângulos formaria um tetraedro representativo do sistema inteiro.

A obtenção de dados de ELL para sistemas empregados em reações de esterificação é bem comum (BENETI *et al.*, 2014, ARDILA *et al.*, 2013, MAZUTTI *et al.*, 2013, MESQUITA *et al.*, 2011, LIU *et al.*, 2008), entretanto dados desse tipo para sistemas com matérias-primas impuras, como a borra ácida, por exemplo, ainda são bastante escassos, devido principalmente à complexidade inerente a tais sistemas, já que se tratam de misturas de vários componentes. Além isso, a incorporação de dados de ELL para o estudo cinético de reações catalisadas enzimaticamente são

ainda mais escassos, o que torna necessária a combinação de tais estudos para descrever uma reação de esterificação para produção de biodiesel catalisada por um sólido fermentado com atividade lipolítica.

Dessa forma, o objetivo principal deste trabalho foi desenvolver um modelo cinético combinado com efeito de sorção para descrever a reação de síntese de ésteres do biodiesel catalisada por sólido fermentado com atividade lipolítica em sistema livre de solvente, utilizando modelagem termodinâmica para prever comportamento das fases do sistema. Para atingir este objetivo, foi necessário:

- Empregar isoterma de Langmuir multicomponente para descrever a sorção dos componentes do meio reacional no sólido fermentado;
- Aplicar uma equação simplificada da cinética de reação de lipase expressando a velocidade de reação em função da composição da fase sorvida.
- Determinar experimentalmente o estado termodinâmico do equilíbrio líquido-líquido do sistema ternário composto pelos ácidos graxos da borra ácida do óleo de soja + etanol + água;
- Determinar experimentalmente o estado termodinâmico do equilíbrio líquido-líquido do sistema ternário composto pelos ésteres etílicos dos ácidos graxos da borra ácida do óleo de soja + etanol + água;
- Realizar modelagem termodinâmica dos dados de equilíbrio líquido-líquido obtidos a partir de dois modelos teóricos, NRTL e UNIQUAC;

Para melhor execução deste trabalho, ele foi dividido em duas etapas, onde a primeira etapa foi a modelagem cinética da reação de esterificação de ácidos graxos da borra ácida do óleo de soja, cujo modelo combina isoterma de Langmuir multicomponente para descrever a sorção dos componentes do meio reacional ao sólido fermentado e uma equação simplificada baseada no mecanismo Pingue-Pongue Bi Bi para descrever a cinética de reação. A segunda etapa refere-se à obtenção de dados experimentais de ELL e a subsequente modelagem termodinâmica dos sistemas “ácidos graxos + etanol + água” e “ésteres etílicos + etanol + água”.

2. ARTIGOS

2.1. ARTIGO 1 – A COMBINED SORPTION AND KINETIC MODEL FOR MULTIPHASIC ETHYL ESTERIFICATION OF FATTY ACIDS FROM SOYBEAN SOAPSTOCK ACID OIL CATALYZED BY A FERMENTED SOLID WITH LIPASE ACTIVITY IN A SOLVENT-FREE SYSTEM

A metodologia, os resultados e a discussão referentes à incorporação do efeito de sorção na equação cinética que descreve a reação de esterificação de ácidos graxos dó óleo ácido da borra de soja catalisada por sólido fermentado com atividade lipolítica em sistema livre de solvente encontram-se descritos no manuscrito que será submetido à revista Fuel.

A combined sorption and kinetic model for multiphasic ethyl esterification of fatty acids from soybean soapstock acid oil catalyzed by a fermented solid with lipase activity in a solvent-free system

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Highlights

First sorption-kinetic model for lipase-catalyzed esterification in solvent-free medium
Composition of sorbed phase described by a multicomponent Langmuir isotherm
Kinetic equation expressed in terms of composition of the sorbed phase
Model adjusted to conversion and phase composition data obtained at 3 molar ratios
Model could be used to guide scale up of biodiesel production using fermented solids

Abstract

A low cost enzymatic catalyst for biodiesel production can be produced by growing a lipase-producing organism in solid-state fermentation and then drying the solids after the fermentation. Recently, we cultivated *Burkholderia cepacia* on a mixture of sugar cane bagasse and sunflower seed meal and used the dried fermented solid to catalyze the esterification of fatty acids with ethanol in a solvent-free system (Soares et al., 2015, Fuel 159: 364–372). During the reaction, up to 30% of the reaction medium sorbed onto the dried fermented solid, with the sorbed medium having a composition different from that of the bulk reaction medium. In the current work, we develop a combined sorption-kinetic model to describe the reaction kinetics in this system. The sorption of the medium components onto the fermented solid follows a multicomponent Langmuir isotherm, while the kinetic equation is expressed in terms of the composition of the sorbed phase. With a single set of parameters, the model adjusted well to the experimental results obtained by Soares et al. for three different ethanol to fatty acid molar ratios (1:1, 1.5:1 and 3:1), not only for the overall conversion profile, but also for the compositions of the bulk reaction medium and the sorbed phase. The model has potential to be used to guide the scale-up of our system.

Keywords: biodiesel, fermented solid, lipase, *Burkholderia cepacia*, ping pong bi bi, multicomponent Langmuir isotherm

1. Introduction

There has been a recent surge of interest in using lipases to catalyze the production of biodiesel, as a "green" alternative to the traditional alkali-catalyzed route, with some large-scale enzymatic biodiesel plants having already been established [1-3] . However, although this enzymatic route yields a higher quality product and cleaner byproducts, thereby simplifying downstream processing [4-7], its widespread commercial implementation is prevented by the high cost of producing and immobilizing the lipases.

Over the last decade, we have been investigating a strategy for reducing the costs of lipase production: lipases can be produced by solid-state fermentation and then the fermented solid can be dried and used directly as the catalyst in either transesterification or esterification reactions [8-12]. This strategy avoids processing steps that are necessary when lipases are produced by submerged fermentation, namely the recovery of the lipases from the fermentation broth and their subsequent immobilization on a suitable support material. These advantages have prompted other researchers to use fermented solids for biodiesel production in transesterification [13,14] and esterification reactions [15].

The costs of lipase-catalyzed biodiesel production can be reduced further by using solvent-free systems. These systems enable the use of smaller reaction vessels, compared to situations in which the substrates are dissolved in solvents such as *n*-heptane [9,12], *n*-hexane [16,17] or *tert*-butanol [13] and also avoid the need for recovery and recycling of the solvent. Recently, we described the first use of fermented solid to catalyze the solvent-free esterification of fatty acids with ethanol, with the fermented solid being produced by growing the bacterium *Burkholderia cepacia* on a mixture of sugarcane bagasse and sunflower seed meal [10].

In a subsequent study, we showed that up to 30% of the total mass of the reaction medium sorbed onto the fermented solid during the process and that this sorbed phase had a composition that was quite different from that of the bulk reaction medium [8]. Since this sorbed phase represents the immediate environment of the lipases held within the fermented solid, these results have implications for the modeling of the enzyme reactor. In fact, a new type of model is necessary, since current models of the kinetics of lipase-catalyzed reactions in solvent-free media do not recognize the existence of a sorbed phase with a unique

composition [18-20] . The aim of the current work was therefore to develop a combined sorption-kinetic model capable of describing the reaction profiles in this system. We did this by using a multicomponent Langmuir isotherm to describe the sorption of the medium components on the fermented solid and a simplified equation for the lipase kinetics that expresses the reaction rate as depending on the composition of the sorbed phase.

2. Mathematical model

2.1. System representation, key assumptions and overall modeling strategy

The system used by Soares et al. [8] consists of a reservoir containing a reaction medium that is pumped through a column packed with air-dried fermented solid that has lipase activity (Fig. 1). The reaction medium contains the two substrates, ethanol (superscript Et) and fatty acids from soybean soapstock acid oil (superscript FA) and the two products, ethyl esters of fatty acids from soybean soapstock acid oil (superscript Es) and water (superscript W). Within this system, part of the reaction medium forms a sorbed phase (subscript S), representing the reaction medium that is sorbed on the solids. The rest of the reaction medium constitutes a bulk phase (subscript B), representing the reaction medium that is free to flow through the system, namely the free liquid within the column and the liquid within the reservoir and tubing. Together, these two parts of the reaction medium compose the global reaction medium (subscript G).

Three key assumptions are made. Firstly, the bulk and sorbed phases remain in a sorption equilibrium described by a multicomponent Langmuir isotherm. This implies that there are no mass transfer limitations between the bulk and sorbed phases. Secondly, a negligibly small amount of reaction occurs in each passage of a volume of reaction medium through the bed, such that the concentrations at the inlet and outlet are very similar. As a consequence of this assumption, the bulk phase can be treated as being well mixed. Thirdly, the lipase in the fermented solid is surrounded by sorbed phase, such that the reaction only occurs in this phase.

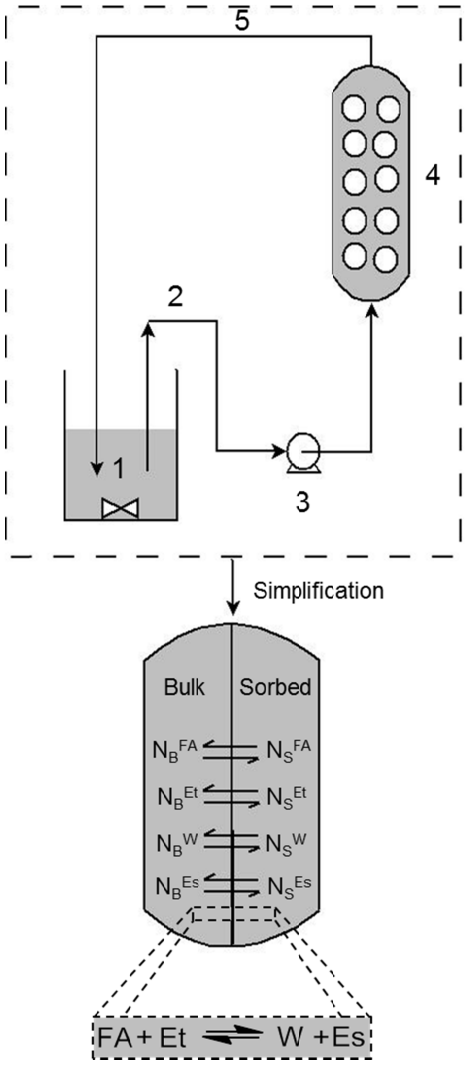


Figure 1. Scheme of real and idealized systems assumed in this study. *N* represents molar compositions, the superscripts FA, Et, W and Es represent fatty acids, ethanol, water and ester, respectively, and the subscripts B and S represent the bulk reaction medium and sorbed phases, respectively. Esterification reaction occurring in the idealized reactor is also identified. Key: (1) reservoir; (2) feed of reaction mixture to the reactor; (3) peristaltic pump; (4) packed-bed reactor containing fermented solids; (5) removal of reaction mixture from the reactor.

The model is solved in two steps (Fig. 2). In the first step, for a given global composition of the reaction medium, the distribution of the components between the bulk and sorbed phases is calculated using the multicomponent Langmuir isotherm. In the second step, the amounts of the components in the sorbed phase are used to calculate the degree to which the reaction progresses over a small time step. This reaction changes the global composition of the reaction medium, such that it is necessary to return to the first step to recalculate the distribution of the components between the bulk and sorbed phases. This cycle of calculations

is repeated until the end of the simulation. The equations used in these two steps are described in the following subsections.

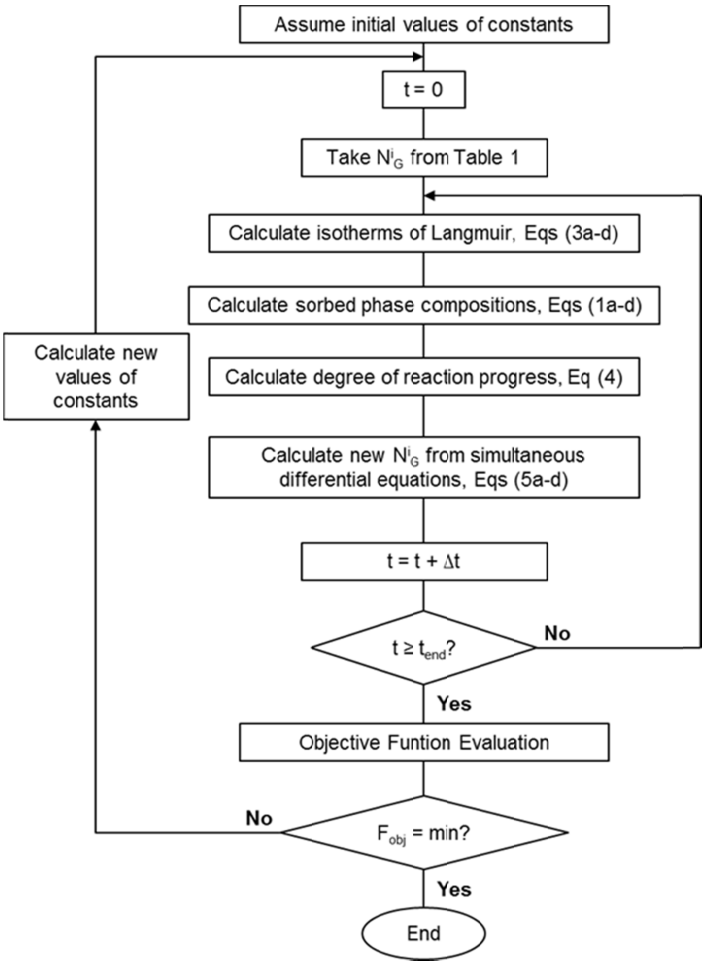


Figure 2. Flowchart of resolution of the kinetic model.

2.2. Step 1: Distribution of components between the bulk and sorbed phases

If the symbol *N* is used to represent the number of moles of a component, with the particular component and the phase being indicated by the appropriate combination of superscript and subscript, then the overall mole balances for the four components of the reaction medium are:

$$N_G^{FA} = N_B^{FA} + N_S^{FA} \quad (1a)$$

$$N_G^{Et} = N_B^{Et} + N_S^{Et} \quad (1b)$$

$$N_G^{W} = N_B^{W} + N_S^{W} \quad (1c)$$

$$N_G^{Es} = N_B^{Es} + N_S^{Es} \quad (1d)$$

The Langmuir isotherm for component *k* in a multicomponent system is given by [21]:

$$N_S^k = \frac{a_k N_B^k}{1 + \sum_{i=1}^{n} b_i N_B^i} \tag{2}$$

where N_S^k is the moles of component *k* in the sorbed phase, N_B^k is the moles of component *k* in the bulk phase, and a_k and b_i are sorption parameters.

Substituting Equation (2) into Equations (1a) to (1d), for all components in the system, gives:

$$N_G^{FA} = N_B^{FA} + \frac{a_{FA} N_B^{FA}}{1 + b_{FA} N_B^{FA} + b_{Et} N_B^{Et} + b_W N_B^W + b_{Es} N_B^{Es}} \tag{3a}$$

$$N_G^{Et} = N_B^{Et} + \frac{a_{Et} N_B^{Et}}{1 + b_{FA} N_B^{FA} + b_{Et} N_B^{Et} + b_W N_B^W + b_{Es} N_B^{Es}} \tag{3b}$$

$$N_G^{W} = N_B^{W} + \frac{a_{W} N_B^{W}}{1 + b_{FA} N_B^{FA} + b_{Et} N_B^{Et} + b_W N_B^W + b_{Es} N_B^{Es}} \tag{3c}$$

$$N_G^{Es} = N_B^{Es} + \frac{a_{Es} N_B^{Es}}{1 + b_{FA} N_B^{FA} + b_{Et} N_B^{Et} + b_W N_B^W + b_{Es} N_B^{Es}} \tag{3d}$$

For a given combination of N_G^{FA}, N_G^{Et}, N_G^W and N_G^{Es}, this nonlinear equation system was solved using the multivariable Newton-Raphson method (*fsolve* function of Matlab®), giving values for N_B^{FA}, N_B^{Et}, N_B^W and N_B^{Es}. Eqs (1a) to (1d) were then used to calculate N_S^{FA}, N_S^{Et}, N_S^W and N_S^{Es}.

2.3. Step 2: Kinetics of lipase-catalyzed reaction

The kinetic equation used is a simplification of the ping-pong bi bi kinetic equation, with the reaction rate depending on the amounts of the reaction components in the sorbed phase:

$$r = \frac{V_{max,f} N_S^{FA} N_S^{Et} - \alpha N_S^W N_S^{Es}}{D_1 N_S^{FA} + D_2 N_S^{Et} + N_S^{FA} N_S^{Et} + D_3 N_S^W + D_4 N_S^{Es}} \tag{4}$$

The overall rates of changes of the components in the global reaction medium are therefore given by:

$$\frac{dN_G^{FA}}{dt} = -r \quad (5a)$$

$$\frac{dN_G^{Et}}{dt} = -r \quad (5b)$$

$$\frac{dN_G^{F}}{dt} = r \quad (5c)$$

$$\frac{dN_G^{Es}}{dt} = r \quad (5d)$$

Equations (5a) to (5d) were solved using the ode45 solver function in the Matlab® software package.

2.4. Initial values and estimation of parameters

The Matlab® function *fminsearch* was used to determine the values of the parameters of the equation giving the best fit to the experimental data of Soares et al. [8] (Fig. 2). The fitting procedure was applied simultaneously to the data from three experiments that started with different molar ratios of ethanol to fatty acid (1:1, 1.5:1 and 3:1). In each experiment, Soares et al. [8] obtained data for the overall conversion, the composition of the bulk reaction medium and the composition of the sorbed phase, at five different times (6, 12, 24, 36 and 48 h).

To simulate a particular experiment, the appropriate initial values of N_G^{FA}, N_G^{Et}, N_G^{W} and N_G^{Es} are taken from Table 1. Using the current values for the Langmuir coefficients (a_{FA}, a_{Et}, a_W, a_{Es}, b_{FA}, b_{Et}, b_W, b_{Es}) and the coefficients of the kinetic equation ($v_{max,f}$, α, D_1, D_2, D_3 and D_4), steps 1 and 2 of the solution strategy are repeated, with integration over 0.1 h intervals in step 2, for 48 h.

Table 1. Global amounts of reaction components in the three experiments that Soares et al. [8] undertook with different initial molar ratios of ethanol to fatty acid*

Initial molar ratio of ethanol to fatty acid	Time (h)	N_G^{FA} (mol)	N_G^{Et} (mol)	N_G^{Es} (mol)	N_G^{W} (mol)
1:1	0	0.360	0.365	0.000	0.026
	6	0.148	0.133	0.144	0.083
	12	0.075	0.057	0.193	0.083
	24	0.053	0.030	0.217	0.056
	36	0.053	0.029	0.218	0.082
	48	0.058	0.027	0.214	0.077
1.5:1	0	0.362	0.491	0.005	0.035
	6	0.155	0.283	0.144	0.130
	12	0.055	0.139	0.225	0.110
	24	0.031	0.091	0.247	0.069
	36	0.029	0.073	0.239	0.072
	48	0.028	0.065	0.238	0.066
3:1	0	0.361	0.981	0.012	0.054
	6	0.214	0.705	0.116	0.190
	12	0.132	0.576	0.193	0.270
	24	0.076	0.503	0.241	0.235
	36	0.049	0.410	0.262	0.215
	48	0.052	0.393	0.260	0.229

* These global values were not reported directly in Soares et al. [8], rather, data were reported for the bulk reaction medium and sorbed phase. These global data represent the sum of these two phases.

In the fitting procedure, at each time that corresponds to a sampling time of the work of Soares et al. [8], the values predicted by the model for the molar percentages (denoted with the symbol *X*, with appropriate superscript and subscript) in the bulk phase are calculated as:

$$X_B^{FA} = \frac{N_B^{FA}}{N_B^{FA} + N_B^{Et} + N_B^{W} + N_B^{Es}} \times 100\% \quad (6a)$$

$$X_B^{Et} = \frac{N_B^{Et}}{N_B^{FA} + N_B^{Et} + N_B^{W} + N_B^{Es}} \times 100\% \quad (6b)$$

$$X_B^{W} = \frac{N_B^{W}}{N_B^{FA} + N_B^{Et} + N_B^{W} + N_B^{Es}} \times 100\% \quad (6c)$$

$$X_B^{Es} = \frac{N_B^{Es}}{N_B^{FA} + N_B^{Et} + N_B^{W} + N_B^{Es}} \times 100\% \quad (6d)$$

Likewise, the values of the molar percentages in the sorbed phase are calculated as:

$$X_S^{FA} = \frac{N_S^{FA}}{N_S^{FA} + N_S^{Et} + N_S^{W} + N_S^{Es}} \times 100\% \tag{7a}$$

$$X_S^{Et} = \frac{N_S^{Et}}{N_S^{FA} + N_S^{Et} + N_S^{W} + N_S^{Es}} \times 100\% \tag{7b}$$

$$X_S^{W} = \frac{N_S^{W}}{N_S^{FA} + N_S^{Et} + N_S^{W} + N_S^{Es}} \times 100\% \tag{7c}$$

$$X_S^{Es} = \frac{N_S^{Es}}{N_S^{FA} + N_S^{Et} + N_S^{W} + N_S^{Es}} \times 100\% \tag{7d}$$

The percentage conversion (C) of the fatty acid into ester is calculated as:

$$C = \left(\frac{N_G^{Es}}{N_{G,o}^{FA}}\right) \times 100\% \tag{8}$$

where $N_{G,o}^{FA}$ is the global moles of fatty acids at the start of the reaction.

The molar percentages and the percentage conversion are used in the calculation of the objective function (F_o):

$$\begin{aligned} F_o = \sum_{1}^{N_E}\sum_{1}^{N_P}[&(X_S^{FA} - x_S^{FA})^2 + (X_S^{Et} - x_S^{Et})^2 + (X_S^{W} - x_S^{W})^2 \\ &+ (X_S^{Es} - x_S^{Es})^2] \\ &+ \sum_{1}^{N_E}\sum_{1}^{N_P}[(X_B^{FA} - x_B^{FA})^2 + (X_B^{Et} - x_B^{Et})^2 + (X_B^{W} - x_B^{W})^2 \\ &+ (X_B^{Es} - x_B^{Es})^2] + \sum_{1}^{N_E}\sum_{1}^{N_P}(C - c)^2 \end{aligned} \tag{9}$$

where the symbol x represents experimentally determined mole fractions, c is the experimentally determined percentage conversion, N_E is the number of experiments (N_E = 3) and N_P is the number of experimental sampling points (N_P = 5).

3. Results

The model adjusted reasonably well to the experimental profiles obtained by Soares et al. [8] for the esterification of fatty acids from soybean soapstock acid oil with ethanol in a solvent-free system, in three different experiments undertaken with different initial molar ratios of ethanol to fatty acid. The fit was acceptable not only for the overall percentage conversion, but also for the compositions of the bulk and sorbed phases. Table 2 shows the root mean square deviations (rmsd) for each medium component in each of the three experiments, while Table 3 shows the optimized values of the model parameters.

Table 2. Rmsd values (%) of model fitting

		rmsd at molar ratio of		
		1:1	**1.5:1**	**3:1**
Overall conversion		8.7	5.0	15.1
Components in bulk phase	Fatty acids	1.6	5.7	5.5
	Ethanol	15.1	11.0	12.7
	Esters	14.5	13.2	3.4
	Water	6.2	12.6	8.2
Components in sorbed phase	Fatty acids	2.2	3.9	3.3
	Ethanol	13.3	25.9	17.0
	Esters	5.9	12.5	11.4
	Water	10.0	12.2	24.9

Table 3. Optimized values of the model parameters

Parameter	Optimized value with units
a_{FA}	0.218 mol
a_{Et}	2.333 mol
a_{W}	3.121 mol
a_{Es}	0.438 mol
b_{FA}	5.02×10^{-5}
b_{Et}	7.901
b_{W}	0.118
b_{Es}	4.1×10^{-4}
$v_{max,f}$	21.309 mol h^{-1}
α	0.517 mol h^{-1}
D_1	2.83×10^{-4} mol
D_2	8.352 mol
D_3	0.002 mol
D_4	8.055 mol

Figure 3 shows the fit of the model to the overall conversion data at the three ethanol to fatty molar ratios, which will be referred hereafter as MR1:1, MR1.5:1 and MR3:1. For MR1:1 and MR1.5:1, the fitted profiles are quite close to the data points over the whole reaction. On the other hand, the fitted profile for MR3:1 is reasonably close to the data points up to 24 h, but then continues to rise, reaching a conversion of about 95% at 48 h. In contrast, the data points rise more slowly, reaching only 84.6% at 48 h. The fact that this discrepancy only occurred for MR3:1 is probably due to the difference in the composition of the sorbed phase: at this molar ratio, the molar percentage of ethanol within the sorbed phase remained above 65% throughout the reaction whereas for MR1:1 and MR1.5:1 the molar percentage of ethanol remained below 30%. The high molar percentage of ethanol in the sorbed phase for MR3:1 may have been sufficient to cause denaturation of the lipases contained in the fermented solid, and this phenomenon is not described by our model.

Figure 4 shows the fit of the model to the data for the composition of the bulk reaction phase in the three experiments undertaken with different initial ethanol to fatty acid molar ratios. As Table 2 shows, all rmsd values were 15% or below. Importantly, the model was able to describe the experimental observation that the major component of the bulk reaction medium is different in the different molar ratios. For MR1:1 and MR1.5:1, the ester is the major component of the bulk medium by 12 h and continues to rise, reaching molar percentages

around 60% by the end of the reaction. On the other hand, for MR3:1, ethanol remains as the major component of the bulk medium throughout the reaction, decreasing from an initial value of 70% to around 40% at the end of the reaction. The model captured this behavior (Fig. 4).

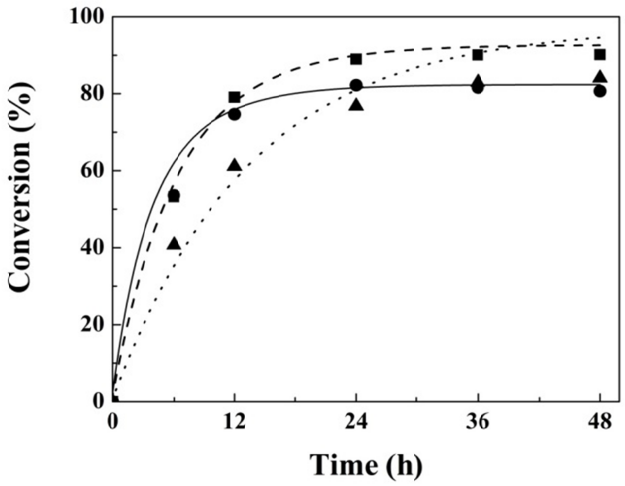


Figure 3. Fit of the model to the data obtained by Soares et al. [8] for overall conversion of fatty acids to ethyl esters, during esterification reactions undertaken with different initial molar ratios of ethanol to fatty acid. Symbols represent experimental data while lines represent the fitted model. Key: Initial ethanol to fatty acid molar ratios of (●, —) 1:1; (■,– – –) 1.5:1; and (▲,·····) 3:1.

Figure 5 shows the fit of the model to the data for the composition of the sorbed phase in the three experiments undertaken with different initial ethanol to fatty acid molar ratios. As Table 2 shows, the fit was not quite as good as for the bulk phase, with the rmsd being around 25% in two cases: ethanol for MR1.5:1 (Fig. 5b) and water for MR3:1 (Fig. 5c). On the other hand, the model was able to describe the experimental observation that the major component of the sorbed phase is different in the different molar ratios. For MR1:1 and MR1.5:1, water is the major component of the sorbed phase by 6 to 12 h, reaching molar percentages around 60% by the end of the reaction. On the other hand, for MR3:1, ethanol remains as the major component of the sorbed phase throughout the reaction, with a value of over 80% at 6 h, decreasing to slightly below 70% at the end of the reaction. Not only did the model capture this behavior, but it also captured the decrease in the ester contents of the sorbed phase with increasing molar ratio of ethanol to fatty acid, with molar percentages after 12 h being around 20% for MR1:1, around 15% for MR1.5:1 and between 5 and 10% for MR3:1 (Fig. 5).

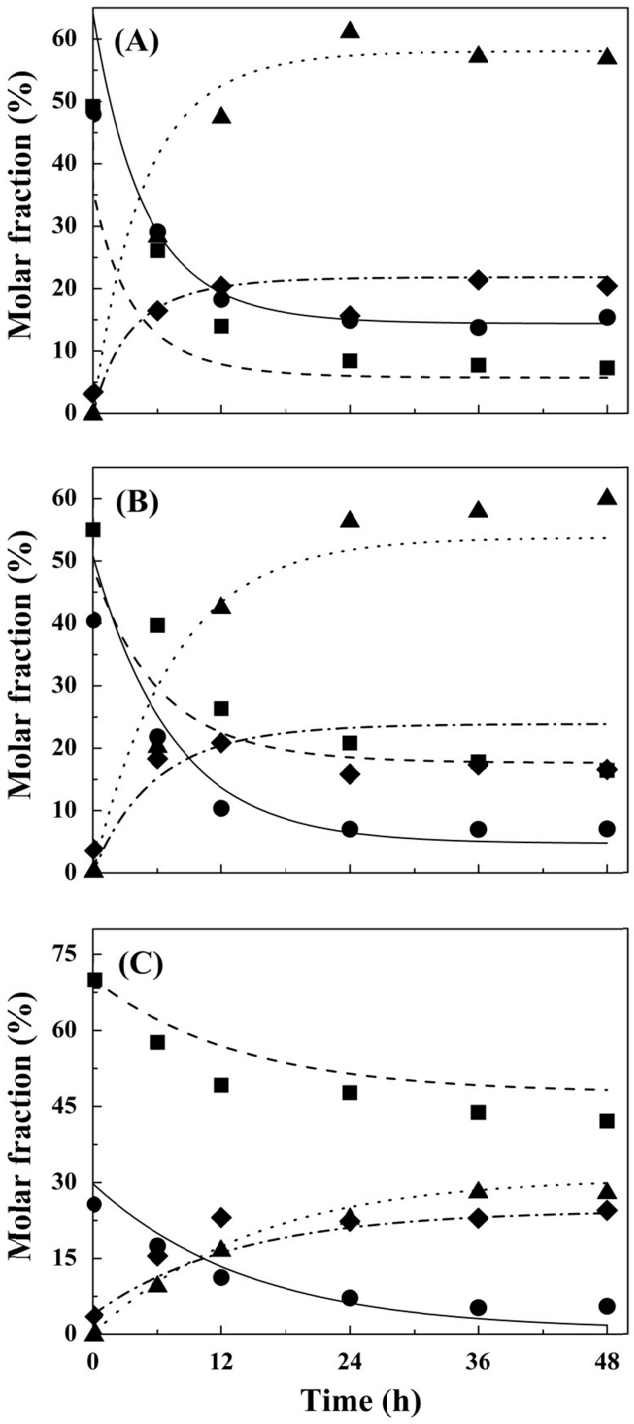


Figure 4. Fit of the model to data obtained by Soares et al. [8] for the composition of the bulk reaction medium. The esterification reactions were undertaken with different initial molar ratios of ethanol to fatty acid: (A) 1:1; (B) 1.5:1; and (C) 3:1. Symbols represent experimental data while lines represent the fitted model. Key: (●, —) fatty acids; (■,– – –) ethanol; (▲,·····) ethyl esters; and (◆,–·–·–) water.

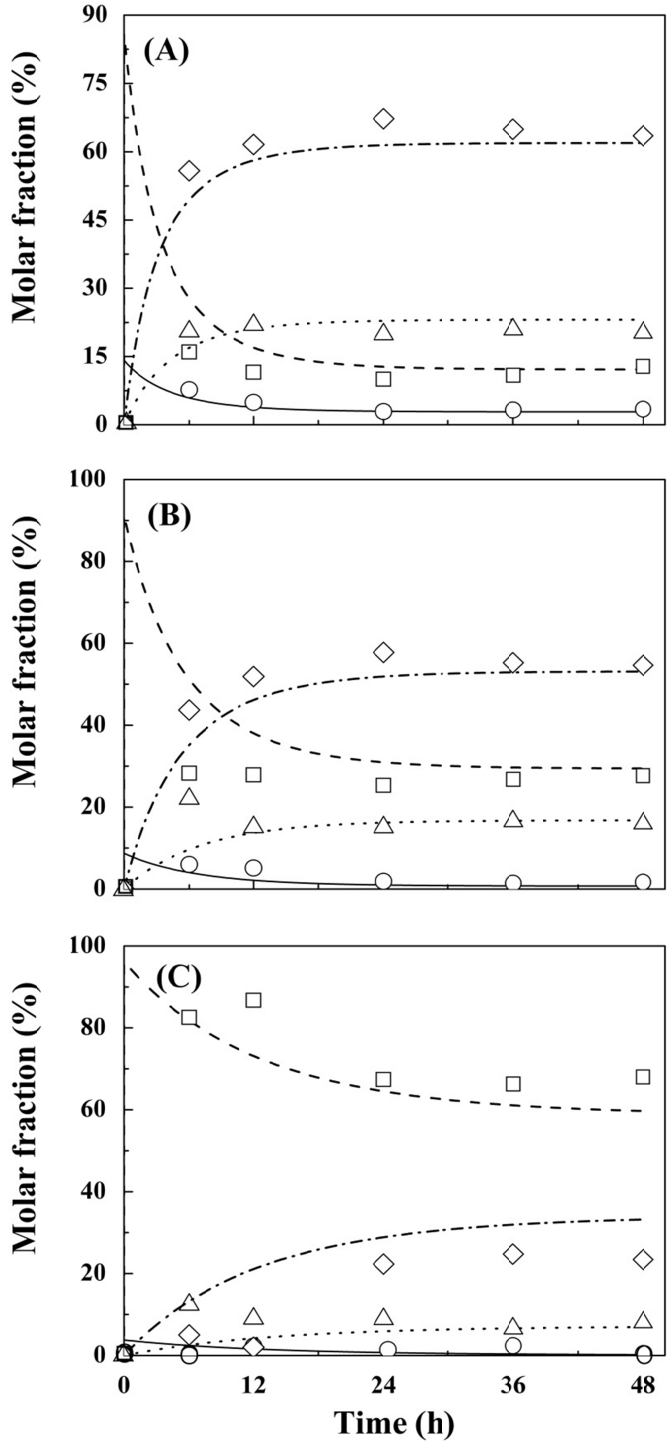


Figure 5. Fit of the model to data obtained by Soares et al. [8] for the composition of the sorbed phase. The esterification reactions were undertaken with different initial molar ratios of ethanol to fatty acid: (A) 1:1; (B) 1.5:1; and (C) 3:1. Symbols represent experimental data while lines represent the fitted model. Key: (○,—) fatty acids; (□,– – –) ethanol; (△,⋯⋯) ethyl esters; and (◇,–·–·–) water.

We undertook a sensitivity analysis with the parameters of both the multicomponent Langmuir sorption equations and the parameters of the kinetic equation. All parameters were varied to 30% below the value obtained in the optimization and to 30% above the value obtained in the optimization. Table 4 shows that five of the parameters had a negligible effect on the fit of the model.

Table 4. Sensitivity of the fitting of the model to the values of the parameters

Parameter	Parameter decreased by 30%		Parameter increased by 30%	
	Sum of squares	% change*	Sum of squares	% change
a_{FA}	6021	46.8	4917	19.9
a_{Et}	4856	18.4	4495	9.6
a_{Es}	4466	8.9	4340	5.8
a_W	4707	14.8	4388	7.0
b_{FA}	4100	0.0002	4100	0.0002
b_{Et}	5093	24.2	4903	19.6
b_{Es}	4100	0.0002	4100	0.0002
b_W	4100	0.0007	4100	0.001
$v_{max,f}$	6198	51.2	5020	22.4
α	4178	1.9	4159	1.4
D_1	4100	0.0002	4100	0.0002
D_2	4780	16.6	4653	13.5
D_3	4100	0.0002	4100	0.0003
D_4	4220	2.9	4195	2.3

* Calculated as the (SS-SSopt)/SSopt × 100%, where SS is the sum of squares obtained with the varied parameter and SSopt is the value of the sum of squares obtained with all parameters optimized (i.e. 4100)

Three of the parameters that had a negligible effect on the fit of the model were parameters in the denominator of the multicomponent Langmuir sorption equation (b_{FA}, b_W and b_{Es}) and two were parameters from the denominator of the kinetic equation (D_1 and D_3). When each of these parameters was set to zero, individually, the percentage change in the sum of squares (compared to that obtained in the original optimization) remained below 0.01%. These five parameters were therefore removed from the model. This resulted in the following set of simplified multicomponent Langmuir sorption equations

$$N_S^{FA} = \frac{a_{FA} N_B^{FA}}{1 + b_{Et} N_B^{Et}} \tag{10a}$$

$$N_S^{Et} = \frac{a_{Et} N_B^{Et}}{1 + b_{Et} N_B^{Et}} \quad (10b)$$

$$N_S^{W} = \frac{a_{W} N_B^{W}}{1 + b_{Et} N_B^{Et}} \quad (10c)$$

$$N_S^{Es} = \frac{a_{Es} N_B^{Es}}{1 + b_{Et} N_B^{Et}} \quad (10d)$$

and the following simplified kinetic equation

$$r = \frac{V_{max,f} N_S^{FA} N_S^{Et} - \alpha N_S^{W} N_S^{Es}}{D_2 N_S^{Et} + N_S^{FA} N_S^{Et} + D_4 N_S^{Es}} \quad (11)$$

When the parameters of this simplified model were determined by fitting the model to the data of Soares et al. [8], their values changed negligibly from the values shown in Table 3. The sum of squares obtained with the simplified model was 4100, compared to the sum of squares of 4100 obtained in the fitting of the complete model.

4. Discussion

Our model makes two contributions in the area of lipase-catalyzed biodiesel production. Firstly, it is the first model to describe the use of fermented solid to catalyze the esterification of fatty acids in a solvent-free medium. Secondly, although there are various mathematical models for the esterification of fatty acids in solvent-free systems by other types of immobilized lipases, our model is the first to recognize the existence of a specific sorbed phase that has a composition different from those of the free liquid phases that are present in the system. In the following subsections, we discuss not only these two contributions but also the limitations and potential of our model.

4.1. Modeling of kinetics of reactions catalyzed by fermented solid with lipase activity

The only previous model describing the use of fermented solid with lipase activity to catalyze reactions in water-restricted media is that of Liu et al. [13], who studied the ethanolysis of

triacylglycerols in the presence of *tert*-butanol. They used the following kinetic equation for the ethanolysis reaction:

$$r = \frac{V_{max}[TG][Et]}{K_M^{TG}[Et]\left(1 + \frac{[Et]}{K_i}\right) + K_M^{Et}[TG] + [TG][Et]} \quad (12)$$

where $[TG]$ is the concentration of triacylglycerol, $[Et]$ is the concentration of ethanol, K_M^{TG} and K_M^{Et} are Michaelis constants for triacylglycerol and ethanol, respectively, while K_i is the constant associated with substrate inhibition by ethanol.

Even though our kinetic equation is for esterification while the equation of Liu et al. [13] is for transesterification, a comparison of Eqs (4) and (12) shows that our approach to modeling is preferable, for two reasons. Firstly, their kinetic equation does not have a term for the reverse reaction in the numerator; this brings the implicit assumption that the reaction is irreversible and will therefore proceed until the limiting substrate has been completely consumed. If this limiting substrate is the triacylglycerol, then this model will always predict a final conversion of 100%. Our equation contains a term for the reverse reaction and can therefore predict final conversions less than 100%. Secondly, their kinetic equation is expressed in terms of the concentrations of the substrates in the bulk reaction medium. However, the fermented solid, which in both their case and ours consists of a 50:50 (dry mass) mixture of sugarcane bagasse and sunflower seed meal, sorbs components from the bulk reaction medium, and the composition of the sorbed phase is quite different from that of the bulk reaction medium [8]. This sorbed phase represents the microenvironment of the lipases held within the fermented solid, so it is preferable to use the concentrations of these components in this sorbed phase in the kinetic equation.

4.2. Recognition of a sorbed phase with a unique composition in solvent-free systems involving immobilized lipases

Two models based on the ping pong bi bi mechanism have previously been proposed for lipase-catalyzed reactions in solvent-free systems that contain different phases: Foresti et al. [19] modeled the esterification of oleic acid with ethanol, by a lipase immobilized on chitosan, whereas Krause and Fieg [18] modeled the hydrolysis of methyl-octanoate, by lipase

B of *Candida antarctica* immobilized on poly-(methyl methacrylate) beads. In both models, the kinetics of the immobilized lipase were expressed in terms of the composition of the organic phase that would be predicted for a liquid-liquid equilibrium based on the global reaction medium in the absence of sorption phenomena. Since neither model recognizes the possibility of the immobilized lipase being in contact with a sorbed phase with a unique composition resulting from specific sorption phenomena, these models are not appropriate for describing reactions catalyzed by fermented solid that have lipase activity. For this type of system, it is necessary to use a combined sorption-kinetic model, such as that developed in the current work.

Our approach to modeling has similarities with models describing the hydrolysis of oils by lipases located at the oil-water interface within an emulsion, insofar as the concentrations of components at the interface can be different from those of the bulk phases in the system. For example, Hermansyah et al. [22] modeled the hydrolysis of triolein by *Candida rugosa* lipase. The sorption of enzyme at the oil-water interface was described by a single-component Langmuir isotherm. On the other hand, the concentrations of the substrates (water and triacylglycerols, diacyglycerols and monoacylglycerols) and the products (diacyglycerols, monoacylglycerols and glycerol) at the interface were described as being directly proportional to the concentrations.

4.3. Possible extensions to our soprtion-kinetic model

Our model could be further improved with the incorporation of two key phenomena: ethanol-related denaturation of the lipases in the fermented solid and phase separation within the reservoir.

Firstly, it is quite possible that the lipase in the fermented solid was denatured by the high ethanol concentrations present in the sorbed phase during the experiment undertaken with an initial ethanol to fatty acid molar ratio of 3:1. This might explain the relatively slow reaction rate: up to 36 h the conversions in this system were lower than those for the ethanol to fatty acid molar ratios of 1.5:1 and 1:1 [8]. In fact, it was for the 3:1 molar ratio that the model adjusted most poorly to the conversion results (see Fig. 3). An expression could therefore be proposed for ethanol-related denaturation of the lipase in the fermented solid.

Secondly, Soares et al. [8] obtained the experimental results shown in Figs 3 to 5 using a well-mixed reservoir. In a further experiment, they obtained a higher conversion at 48 h by allowing the organic and aqueous phases of the bulk reaction medium to separate in the reservoir (i.e. by operating the reservoir without agitation) and recycling only the organic phase back to the packed-bed reactor containing the fermented solid. We could describe this mode of operation by restricting our kinetic-sorption model to the packed-bed reactor and describing the phase equilibrium in the reservoir using a model based on the liquid-liquid equilibrium data that we previously obtained for this particular system (fatty acids from soybean soapstock acid oil + ethanol + water + ethyl esters of fatty acids from soybean soapstock acid oil) [23].

Finally, our assumption that mass transfer is not limiting in the system might not be correct. If so, then the constants in Eq. (4) would lump together the kinetic and mass transfer effects, rather than being true kinetic constants [24]. However, even if this is the case, it may be preferable to accept that they are lumped parameters, rather than trying to develop a model with explicit mass transfer equations. A model with separate mass transfer equations would require detailed knowledge of the phases within the fermented solid and the intrinsic kinetics of the lipase held within the solid. Experimental difficulties would arise because there may be more than one sorbed phase, since the fermented solid contains two components with quite different physical properties: sugarcane bagasse is hydrophilic, while the sunflower seed meal is oily and, therefore, hydrophobic [8]. A further difficulty is that the intrinsic kinetics cannot be determined in isolation of mass transfer effects by studying reactions undertaken with free lipase: the kinetics of free and immobilized lipases are quite different, since immobilization affects the conformation of the lipase [25].

5. Conclusion

In the current work, we developed a combined sorption-kinetic model to describe the esterification of fatty acids with ethanol in a solvent-free system, with “lipolytic fermented solid” as the catalyst. The model adjusted well to the time course data that Soares et al. [8] obtained for, firstly, overall conversion and, secondly, the compositions of the bulk reaction phase and the phase sorbed on the fermented solid. With future modifications, such as the

inclusion of ethanol-related denaturation of the lipase and of phase separation in the system reservoir, our model could become a useful tool for guiding the scale-up of our system.

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2.2. ARTIGO 2 – LIQUID–LIQUID EQUILIBRIUM DATA AND THERMODYNAMIC MODELING FOR SYSTEMS RELATED TO THE PRODUCTION OF ETHYL ESTERS OF FATTY ACIDS FROM SOYBEAN SOAPSTOCK ACID OIL

A metodologia, os resultados e a discussão referentes à obtenção experimental de diagramas ternários para o sistema “ácidos graxos + etanol + água + ésteres etílicos”, bem como sua modelagem termodinâmica encontram-se descritos no artigo publicado no periódico Fuel.

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Liquid–liquid equilibrium data and thermodynamic modeling for systems related to the production of ethyl esters of fatty acids from soybean soapstock acid oil

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HIGHLIGHTS

- We measured LLE for the ethanol-based biodiesel systems.
- Soybean soapstock acid oil + FAEE + (ethanol + water) were measured.
- Tie lines and binodal curves were measured for all systems.
- The experimental data were well modeled using NRTL and UNIQUAC models.

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ABSTRACT

We obtained liquid–liquid equilibrium (LLE) data for the systems involved in biodiesel production from a low-value feedstock, namely fatty acids from soybean soapstock acid oil (FA-SSAO), at 303.2 and 323.2 K. The systems studied were water + ethanol + FA-SSAO and water + ethanol + fatty acid ethyl esters (EE-SSAO). The mass fractions of the compounds in equilibrium in the two phases were determined analytically, whereas the binodal curves were obtained by the cloud-point method. The increase in temperature from 303.2 and 323.2 K slightly increased the miscibility of the EE-SSAO system. We found that ethanol is more soluble in water than in either FA-SSAO or EE-SSAO, and that an increase in ethanol content in either the FA-SSAO-rich phase or the EE-SSAO-rich phase enhances water solubility in these phases. The experimental data were satisfactorily modeled by the NRTL and UNIQUAC approaches.

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1. Introduction

Biodiesel is becoming an increasingly important renewable fuel, not only because it is biodegradable and environmentally friendly when compared to fossil fuels, but also because its use reduces the emission of greenhouse gases [1]. However, in Brazil commercial production of biodiesel utilizes relatively expensive starting materials, such as refined soybean oil. As a result, it is economically uncompetitive with petrodiesel and it is only used due to legislation that prescribes a minimum content of 7% (as of November 2014) in commercialized diesel. The use of low-value feedstock, such as animal fat from sewage and residual oil of domestic, industrial and commercial origin, could improve the economic viability of biodiesel [2]. One residual oil that could be used to reduce the costs of biodiesel production is soybean soapstock acid oil (SSAO), which is a mixture of oil and fatty acids that is produced during the refining of soybean oil.

Since soybean soapstock acid oil contains significant amounts of free fatty acids (about 50%), the most appropriate route for obtaining biodiesel with this feedstock is hydroesterification, since the free fatty acids would form soaps if transesterification were attempted [2–5]. In hydroesterification, the triacylglycerols in the SSAO are hydrolyzed in subcritical water, with a pure stream of free fatty acids being obtained by distillation. These free fatty acids are then esterified with a short chain alcohol, such as methanol or ethanol. In the esterification reaction, it is necessary to remove the water that is produced in order to favor ester production. Also, water is used in the washing step. Moreover, the non-reacted ethanol must be efficiently removed to avoid co-solvent effects

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that hamper the biodiesel recovery. Consequently, in both the reaction itself and the subsequent purification of biodiesel, it is necessary to know the phase behavior of the components involved, mainly liquid–liquid equilibrium (LLE) data.

Although various authors have obtained LLE data for systems related to biodiesel production [6–13], none of them have used a low-cost feedstock, such as fatty acids from soybean soapstock acid oil (FA-SSAO). The aim of the current work was therefore to provide LLE data for components involved in biodiesel production through esterification of FA-SSAO. Since ethanol is less toxic than methanol and is also abundantly available in Brazil, it was chosen as the alcohol. Two systems were characterized, namely water(1) + ethanol(2) + FA-SSAO(3) and water(1) + ethanol(2) + EE-SSAO(4) (ethyl esters obtained from soybean soapstock acid oil). We determined the ethanol distribution coefficient between the two liquid phases, the selectivity of water and the selectivity of non-polar compounds (FA-SSAO, EE-SSAO). Data were obtained at both 303.2 and 323.2 K.

2. Materials and methods

2.1. Materials

Fatty acids from soybean soapstock acid oil (FA-SSAO) and the corresponding ethyl esters (EE-SSAO) were obtained according to Soares et al. [2]. In their study, they obtained only 92% of conversion to esters; we increased this value to higher than 98% by using sulfuric acid (Sigma–Aldrich) in an acid-catalyzed esterification (4 h, 353 K). The fatty acid composition of soybean soapstock acid oil was determined by gas chromatography [14] and is shown in Table 1. Ethanol (>99.5% purity from Sigma–Aldrich) and deionized water were used without further treatment.

2.2. LLE apparatus and procedures

The LLE measurements were performed based on the work of Kanda et al. [15], with a few modifications for the experimental procedure. The experiments were carried out in a jacketed glass vessel, of 30 mL total volume, that was attached to a thermostatic bath (Tecnal TE-184) and agitated using a magnetic stirrer (Fisatom 752). Binodal curves of ternary systems were determined based on the cloud point transition, under isothermal conditions. The binodal curves were obtained at 303.2 and 323.2 K and represented in mass fraction (w), for both systems.

The binodal curves for the water(1) + ethanol(2) + FA-SSAO(3) system were obtained as follows. For the FA-SSAO-rich phase, a known mass of FA-SSAO was added to the cell and then water was added dropwise with a syringe until the cloud point was visible for 3–4 min. The syringe was then weighed on an analytical balance (Adan, model AAA200, accurate to 0.0001 g) to determine the mass of water that had been added to the cell. In this manner, it was possible to calculate the mass fractions of all components at the cloud point. Ethanol was then added dropwise with a syringe until the turbidity disappeared. The syringe with ethanol was weighed to determine the mass added to the system. Again, it was possible to recalculate the mass fractions of all components. For the water-rich phase, the procedure was similar. However, instead of FA-SSAO, a known mass of water was added to the cell, and FA-SSAO was added dropwise with a syringe until the mixture became turbid and remained so for several minutes. Ethanol was then added until the turbidity disappeared.

The binodal curves for the water(1) + ethanol(2) + EE-SSAO(4) system were obtained using the same procedure as that described above for the determination of binodal curves for the water(1) + ethanol(2) + FA-SSAO(3) system. For the EE-SSAO-rich phase, a known mass of EE-SSAO was added to the cell and water was added dropwise with a syringe until the cloud point was visible. Ethanol was then added dropwise with a syringe until the turbidity disappeared. In the case of the water-rich phase, a known mass of water was added to the cell and EE-SSAO was added dropwise with a syringe until the cloud point was visible. Ethanol was then added dropwise with a syringe until the turbidity disappeared.

For determining experimental tie lines, mixtures with compositions within the immiscibility region were prepared directly inside the equilibrium cells. Each component was weighed using an analytical balance. Components were added in the order of increasing volatility: FA-SSAO, water, and then ethanol for the first system and EE-SSAO, water, and then ethanol for the second system. The system was mixed at approximately 800 rpm with a magnetic stirrer for 4 h to allow good contact between the phases and was then left to rest for 20 h until complete separation into two clean liquid phases. Preliminary experiments showed that the agitation and separation times were sufficient for the system to reach equilibrium.

2.3. Analytical methods

Samples were obtained separately from each liquid phase after the equilibrium was reached. The water content of each phase was then determined by the Karl-Fischer method (TitroLine® 7500 KF) according to the AOCS method [16].

To determine the percent acidity, samples were treated according to the official method [17]. The acidity content in each phase was calculated as:

$$\text{acidity (wt\%)} = \frac{V \times M_{NaOH} \times 277.3}{SW} \quad (1)$$

where V is the volume (L) of NaOH used, M_{NaOH} is the molarity of the NaOH solution (mol L^{-1}), 277.3 is the average molecular weight

Table 1
Characterization[a] of free fatty acids obtained from hydrolysis of soybean soapstock acid oil (FA-SSAO) in subcritical water and their ethyl esters (FAEE), respectively.

Fatty acid (FA)[b]	% By mass of free FA in FA-SSAO	FA molar mass (g mol^{-1})	FAEE[c] molar mass (g mol^{-1})
Palmitic (C16:0)	16.5	256.42	284.48
Stearic (C18:0)	4.2	284.48	312.50
Oleic (C18:1)	33.4	282.46	310.51
Linoleic (C18:2)	44.2	280.45	308.50
Others	1.7	–	–
Average molar mass (g mol^{-1})		277.3	305.32
Other parameters			
FA-SSAO acid value (mg KOH g^{-1})		195.5	
FA-SSAO saponication value (mg KOH g^{-1})		199	
EE-SSAO purity (wt%)		>98%	

[a] Adapted from Soares et al. [2].
[b] In Cx:y, x = number of carbons and y = number of double bonds.
[c] FAEE represents the pure fatty acid ethyl esters.

of the free fatty acids (g mol^{-1}) used in this study and *SW* is the sample weight (g).

Ethyl ester contents in each phase were determined using a GC-2010 (Shimadzu Co., Kyoto, Japan) equipped with an AOC20i auto injector, a hydrogen flame ionization detector and a SGE HT-5 capillary column (internal diameter 0.32 mm, length 25 m and film thickness 0.1 mm). Prepared sample (5–6 mg) was diluted in 1 mL of an internal standard solution of methylheptadecanoate (1 mg mL^{-1}) in *n*-heptane. Then, 1 μL was injected, with a split ratio of 1:20, using N_2 as the carrier gas. The injector and detector were set at 250 °C. The oven program was as follows: 120 °C for 2 min, heating at 10 °C min^{-1} to 180 °C, holding at 180 °C for 3 min, heating at 5 °C min^{-1} to 230 °C, 230 °C maintained for 2 min. Peaks in the chromatograms were identified by comparison of the retention times with a standard solution. The ester content (in mass percent) was determined relative to the peak area of the internal standard.

The ethanol content (wt%) was obtained through the mass balance for each liquid phase using the water content and the fatty acid (FA-SSAO) or fatty acid ethyl ester (EE-SSAO) content, determined as described above.

2.4. Partition coefficients and selectivity

The ethanol partition (distribution) coefficients (K_1 and K_2) were calculated as:

$$K_1 = \frac{w_2^{SS}}{w_2^{W}} \tag{2}$$

$$K_2 = \frac{w_2^{EE}}{w_2^{W'}} \tag{3}$$

where w_2^{SS} and w_2^{W} are the mass fractions of ethanol in the FA-SSAO-rich phase and the water-rich phase, respectively, for the water(1) + ethanol(2) + FA-SSAO(3) system, while w_2^{EE} and $w_2^{W'}$ are the mass fractions of ethanol in the EE-SSAO-rich phase and the water-rich phase, respectively, for the water(1) + ethanol(2) + EE-SSAO(4) system.

Table 2
Binodal data for the system water(1) + ethanol(2) + FA-SSAO(3).

303.2 K			323.2 K		
w_1	w_2	w_3	w_1	w_2	w_3
0.027	0.000	0.973	0.039	0.000	0.961
0.075	0.171	0.754	0.051	0.108	0.841
0.086	0.237	0.677	0.074	0.169	0.757
0.122	0.294	0.584	0.097	0.240	0.662
0.152	0.352	0.497	0.109	0.262	0.628
0.170	0.377	0.454	0.131	0.306	0.564
0.191	0.402	0.407	0.169	0.354	0.478
0.208	0.419	0.373	0.187	0.373	0.440
0.218	0.429	0.353	0.206	0.393	0.402
0.227	0.437	0.336	0.223	0.406	0.371
0.234	0.445	0.321	0.230	0.411	0.360
0.242	0.452	0.306	0.237	0.429	0.334
0.251	0.458	0.291	0.246	0.438	0.317
0.258	0.461	0.281	0.251	0.442	0.307
0.268	0.472	0.260	0.273	0.455	0.272
0.274	0.478	0.248	0.276	0.461	0.263
0.282	0.480	0.238	0.289	0.463	0.249
0.285	0.487	0.228	0.295	0.471	0.234
0.293	0.487	0.219	0.302	0.477	0.221
0.294	0.493	0.213	0.329	0.487	0.184
0.297	0.495	0.208	0.357	0.510	0.134
0.300	0.497	0.203	0.374	0.527	0.099
0.302	0.500	0.198	0.384	0.549	0.066
0.310	0.505	0.186	0.384	0.556	0.060
0.303	0.513	0.183	0.295	0.676	0.030
0.959	0.000	0.041	0.976	0.000	0.024

Standard uncertainties *u* are: $u(T) = 0.5$ K and $u(w) \leq 0.05$.

For the water(1) + ethanol(2) + FA-SSAO(3) system, the selectivity of FA-SSAO (S_{SS}) represents its preference to solubilize ethanol or water,

$$S_{SS} = \frac{K_1}{\left(\frac{w_1^{SS}}{w_1^{W}}\right)} \tag{4}$$

whereas the selectivity of water (S_W) represents its preference to solubilize either ethanol or FA-SSAO,

$$S_w = \frac{K_1^{-1}}{\left(\frac{w_3^{W}}{w_3^{SS}}\right)} \tag{5}$$

In these equations, w_1^{SS} and w_1^{W} are the mass fractions of water in the FA-SSAO-rich phase and in the water-rich phase, respectively, while w_3^{W} and w_3^{SS} are the mass fractions of FA-SSAO in the water-rich phase and in the FA-SSAO-rich phase, respectively.

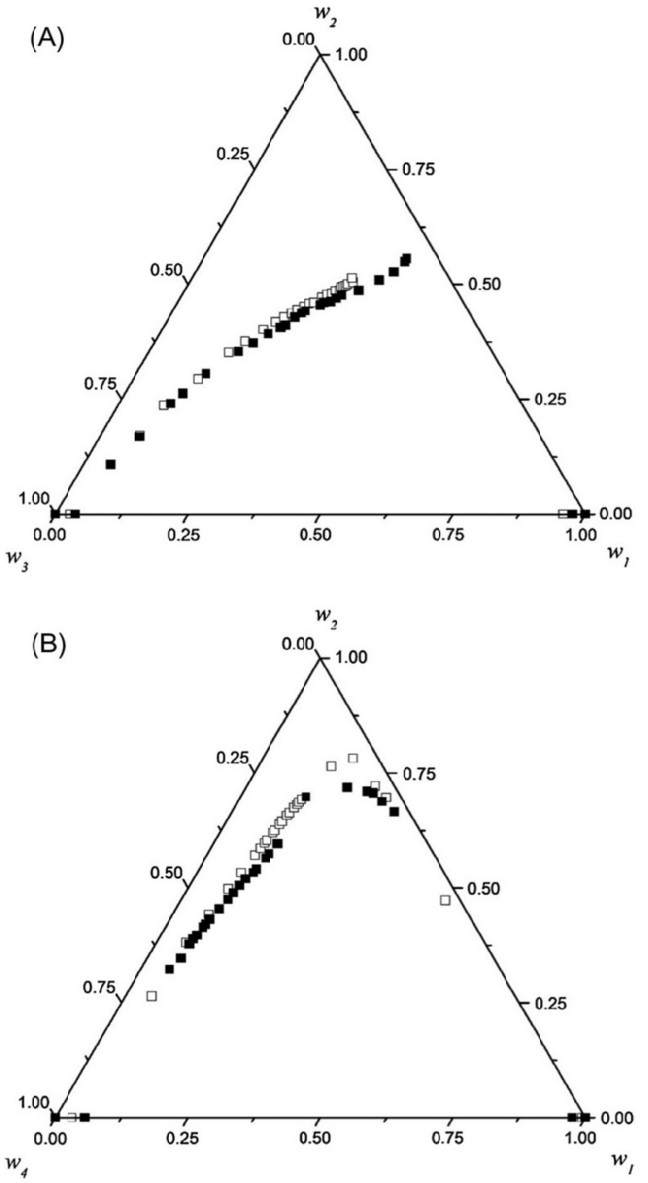


Fig. 1. Experimental binodal curves for the systems (A) water(1) + ethanol(2) + FA-SSAO(3) and (B) water(1) + ethanol(2) + EE-SSAO(4), at $T = 303.2$ K (□) and $T = 323.2$ K (■).

Table 3
Binodal data for the system of water(1) + ethanol(2) + EE-SSAO(4).

303.2 K			323.2 K		
w_1	w_2	w_4	w_1	w_2	w_4
0.032	0.000	0.968	0.057	0.000	0.943
0.050	0.264	0.686	0.054	0.323	0.623
0.055	0.382	0.563	0.064	0.347	0.589
0.069	0.442	0.489	0.064	0.378	0.559
0.077	0.498	0.425	0.065	0.389	0.546
0.084	0.533	0.383	0.068	0.398	0.535
0.091	0.571	0.338	0.071	0.415	0.515
0.094	0.586	0.320	0.073	0.421	0.506
0.096	0.597	0.307	0.075	0.432	0.493
0.097	0.604	0.299	0.083	0.454	0.463
0.100	0.620	0.280	0.088	0.475	0.437
0.102	0.625	0.273	0.091	0.489	0.420
0.105	0.638	0.257	0.094	0.506	0.400
0.106	0.645	0.249	0.099	0.520	0.381
0.108	0.658	0.234	0.107	0.533	0.360
0.110	0.663	0.227	0.109	0.541	0.350
0.113	0.675	0.213	0.115	0.565	0.321
0.115	0.682	0.204	0.117	0.574	0.310
0.116	0.686	0.198	0.122	0.596	0.282
0.119	0.692	0.189	0.124	0.698	0.178
0.139	0.765	0.097	0.192	0.719	0.089
0.172	0.782	0.047	0.233	0.710	0.057
0.243	0.721	0.036	0.247	0.707	0.047
0.276	0.696	0.028	0.272	0.688	0.039
0.500	0.473	0.028	0.306	0.666	0.028
0.995	0.000	0.005	0.976	0.000	0.024

Standard uncertainties u are: $u(T) = 0.5$ K and $u(w) \leqslant 0.05$.

For the water(1) + ethanol(2) + EE-SSAO(4) system, the selectivity of EE-SSAO (S_{EE}) represents its preference to solubilize ethanol or water,

$$S_{EE} = \frac{K_2}{\left(\frac{w_3^{W'}}{w_3^{SS}}\right)} \tag{6}$$

whereas the selectivity of water (S'_W) represents its preference to solubilize either ethanol or EE-SSAO,

$$S'_W = \frac{K_2^{-1}}{\left(\frac{w_4^{W'}}{w_4^{EE}}\right)} \tag{7}$$

In these equations, $w_4^{W'}$ and w_4^{EE} represent the mass fractions of EE-SSAO in the water-rich phase and in the EE-SSAO-rich phase, respectively, while w_1^{EE} and $w_1^{W'}$ represent the mass fractions of water in the EE-SSAO-rich phase and in the water-rich phase, respectively.

In order to verify the quality of the LLE data obtained in this work, we used the Hand [18] correlation, defined by the following equations,

$$\log_{10}\left(\frac{w_2^W}{w_1^W}\right) = a_H \log_{10}\left(\frac{w_2^{SS}}{w_3^{SS}}\right) + b_H \tag{8}$$

$$\log_{10}\left(\frac{w_3^{EE}}{w_4^{EE}}\right) = a_H \log_{10}\left(\frac{w_2^W}{w_1^W}\right) + b_H \tag{9}$$

and the Othmer–Tobias [19] correlation, defined by,

$$\log_{10}\left(\frac{1-w_1^W}{w_1^W}\right) = a_{OT} \log_{10}\left(\frac{1-w_3^{SS}}{w_3^{SS}}\right) + b_{OT} \tag{10}$$

$$\log_{10}\left(\frac{1-w_4^{EE}}{w_4^{EE}}\right) = a_{OT} \log_{10}\left(\frac{1-w_1^W}{w_1^W}\right) + b_{OT} \tag{11}$$

where a and b (with subscripts) represent slopes and intercepts, respectively.

The algorithm of Ferrari et al. [20] was used to calculate the LLE and estimate the parameters of the thermodynamic models. In this algorithm, the LLE calculations are performed based on the phase stability test by analyzing the Gibbs surface tangent plane

Table 4
Liquid–liquid equilibrium data in mass fraction (w) for water(1) + ethanol(2) + FA-SSAO(3).

				Liquid–liquid equilibrium									
T	Overall compositions			FA-SSAO-rich phase			Water-rich phase						
(K)	w_1	w_2	w_3	w_1	w_2	w_3	w_1	w_2	w_3	K_1	S_{SS}	K_2	S_w
303.2	0.438	0.124	0.438	0.016	0.103	0.881	0.855	0.139	0.006	0.741	39.6	1.35	183.6
	0.407	0.186	0.407	0.037	0.130	0.833	0.763	0.229	0.008	0.568	11.9	1.76	180.8
	0.364	0.268	0.368	0.074	0.149	0.777	0.637	0.350	0.013	0.427	3.67	2.34	133.3
	0.333	0.334	0.333	0.062	0.203	0.735	0.532	0.453	0.015	0.448	3.87	2.23	109.9
323.2	0.438	0.124	0.438	0.029	0.074	0.897	0.777	0.216	0.007	0.339	8.99	2.95	392.0
	0.415	0.170	0.415	0.02	0.081	0.899	0.763	0.230	0.007	0.351	13.2	2.85	341.1
	0.353	0.295	0.352	0.066	0.198	0.736	0.594	0.396	0.010	0.502	4.53	1.99	151.3
	0.333	0.335	0.332	0.086	0.243	0.671	0.560	0.426	0.014	0.570	3.71	1.76	88.7

Standard uncertainties u are: $u(T) = 0.5$ K and $u(w) \leqslant 0.002$.

Table 5
Liquid–liquid equilibrium data in mass fraction (w) for water(1) + ethanol(2) + EE-SSAO(4).

				Liquid–liquid equilibrium									
T	Overall compositions			FAEE-rich phase			Water-rich phase						
(K)	w_1	w_2	w_4	w_1	w_2	w_4	w_1	w_2	w_4	K_1	S_{FAEE}	K_2	S_w
303.2	0.366	0.280	0.354	0.008	0.101	0.891	0.619	0.379	0.002	0.267	20.4	3.74	1666.9
	0.307	0.380	0.313	0.009	0.110	0.881	0.477	0.518	0.005	0.213	11.4	4.69	879.2
	0.239	0.521	0.240	0.020	0.120	0.860	0.329	0.654	0.017	0.184	3.11	5.43	271.6
	0.160	0.600	0.240	0.022	0.136	0.842	0.196	0.770	0.034	0.176	1.56	5.66	139.0
323.2	0.439	0.122	0.439	0.007	0.028	0.965	0.767	0.233	0.000	0.120	13.11	8.36	26877.5
	0.389	0.222	0.389	0.007	0.059	0.934	0.625	0.374	0.001	0.157	13.6	6.37	8495.9
	0.349	0.312	0.349	0.007	0.087	0.906	0.528	0.470	0.002	0.184	13.2	5.43	2457.5
	0.301	0.398	0.301	0.009	0.109	0.882	0.445	0.550	0.005	0.197	9.43	5.08	953.3

Standard uncertainties u are: $u(T) = 0.5$ K and $u(w) \leqslant 0.002$.

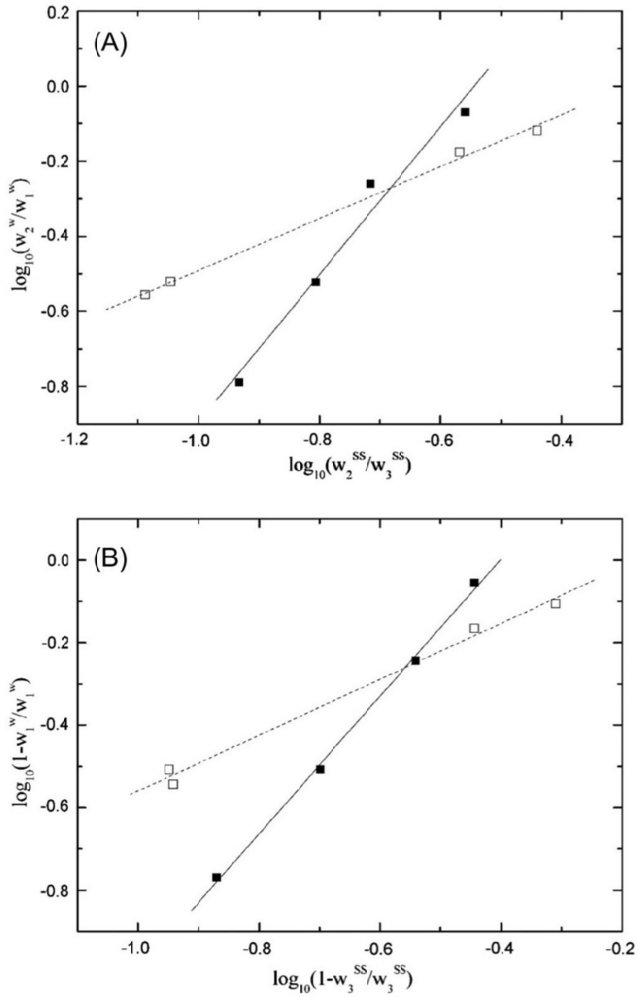


Fig. 2. Hand (A) and Othmer–Tobias correlations (B) for the system water(1) + ethanol(2) + FA-SSAO(3) at T = 303.2 K (–■–) and T = 323.2 K (···□··).

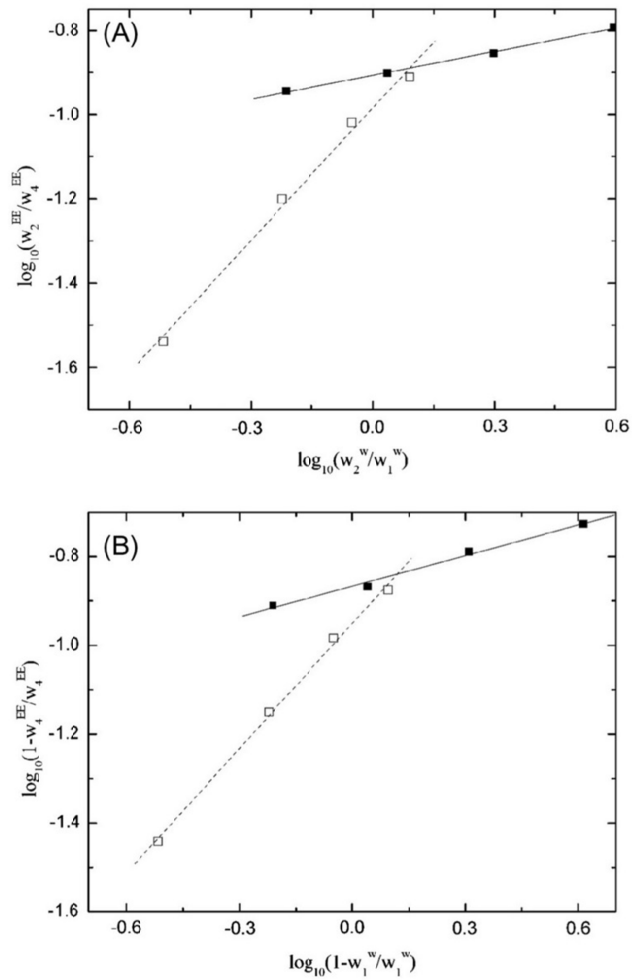


Fig. 3. Hand (A) and Othmer–Tobias correlations (B) for the system water(1) + ethanol(2) + EE-SSAO(4) at T = 303.2 K (–■–) and T = 323.2 K (···□··).

distance; once an instability is identified, the LLE calculation is performed by applying the liquid–liquid flash algorithm. After calculating the LLE, one phase is tested again; if instability is again identified, then the set of parameters that is being used (from the optimization algorithm) is dropped by penalizing the objective function (a barrier method). This strategy avoids the obtaining of a set of inconsistent parameters that gives a wrong number of phases in equilibrium [20]. The same procedure of penalizing the objective function is applied if the stability test identifies a homogeneous region (i.e. a single phase) for a given tie line. Therefore, the objective function for the parameter estimation of the NRTL and UNIQUAC models used in this work can be formulated as:

$$\min F_{\text{obj}} = \sum_{k}^{NT}\sum_{j}^{NP}\sum_{i}^{nc}\frac{\left(w_{ijk}^{calc} - w_{ijk}^{\exp}\right)^2}{\sigma_{ij}^2} \tag{12}$$

where w_{ijk}^{calc} and $w_{ijk}^{\exp}$ are the calculated and experimental mass fractions, respectively, of component i in the phase j related to tie line k, NT is the total number of tie lines, NP is the total number of phases, nc is the number of components in the system and σ_{ij}^2 is the variance of component i in the experimental phase j.

The objective function (Eq. (12)) was minimized using the strategy of Ferrari et al. [16]: the Particle Swarm Optimization algorithm was used to obtain initial guesses for the parameters and these initial guesses were then used in the Nelder–Mead Simplex method [21] to find the final optimized values of the binary parameters.

Table 6
Hand and Othmer–Tobias coefficients (a, b) and linear coefficients of determination (R^2) for the systems studied.

T	Hand			Othmer–Tobias		
(K)	a_H	b_H	R^2	a_{OT}	b_{OT}	R^2
Water(1) + ethanol(2) + FA-SSAO(3)						
303.2	1.964	1.070	0.972	1.666	0.669	0.998
323.2	0.690	0.199	0.997	0.678	0.118	0.992
Water(1) + etanol(2) + EE-SSAO(4)						
303.2	0.188	−0.907	0.997	0.230	−0.867	0.992
323.2	1.047	−0.985	0.994	0.937	−0.951	0.997

In this study, the root mean square deviation (*rmsd* (%)) was used to evaluate the correlation between the model predictions and the experimental data:

$$rmsd\ (\%) = 100\sqrt{\frac{\sum_{k=1}^{NT}\sum_{j=1}^{NP}\sum_{i=1}^{nc}\left(w_{ijk}^{calc} - w_{ijk}^{\exp}\right)^2}{NT \times NP \times nc}} \tag{13}$$

3. Results and discussion

We obtained, for the first time, experimental binodal curves for two systems related to the production of biodiesel from the fatty

acids of a low-value feedstock, soybean soapstock acid oil. These two systems are water(1) + ethanol(2) + fatty acids from soybean soapstock acid oil (FA-SSAO)(3) and water(1) + ethanol(2) + ethyl esters from soybean soapstock acid oil (EE-SSAO)(4). The results for the first system are given in Table 2 and Fig. 1A, for both 303.2 K and 323.2 K, at a pressure ≈0.92 bar (average atmospheric pressure registered in the city of Curitiba). The phase diagram for this system shows that the 20 K increase in temperature had little effect on the area of the heterogeneous region (LLE); in other words, in this system the solubility of the components was essentially independent of temperature over the range studied. The mixtures water + ethanol and FA-SSAO + ethanol were soluble in all proportions. The solubility of water in the FA-SSAO-rich phase was higher than that of FA-SSAO in the water-rich phase.

Regarding the ternary system water(1) + ethanol(2) + ethyl esters from soybean soapstock acid oil (EE-SSAO)(4), the solubility of this ternary system was improved by the increase in temperature from 303.2 K to 323.2 K, as shown by the decrease of the two-phase area in Fig. 1B and by the values in Table 3 (at the same pressure mentioned above). A similar behavior in terms of solubility was obtained by Ardila et al. [8] for the system soybean oil biodiesel + ethanol + water, where an increase by 10 K decreased the area of the two-liquid phase region.

Tables 4 and 5 present the experimental tie line values measured for the systems water(1) + ethanol(2) + FA-SSAO(3) and water(1) + ethanol(2) + EE-SSAO(4), at 303.2 and 323.2 K. Since the feedstock of interest is a complex mixture of fatty acids and the biodiesel produced is a complex mixture of ethyl esters, the equations of Hand and of Othmer–Tobias were used to demonstrate the consistency of the experimental tie-line data. The high values of the coefficients of determination (>0.97) found for both the Hand and Othmer–Tobias equations (Figs. 2 and 3; Table 6)

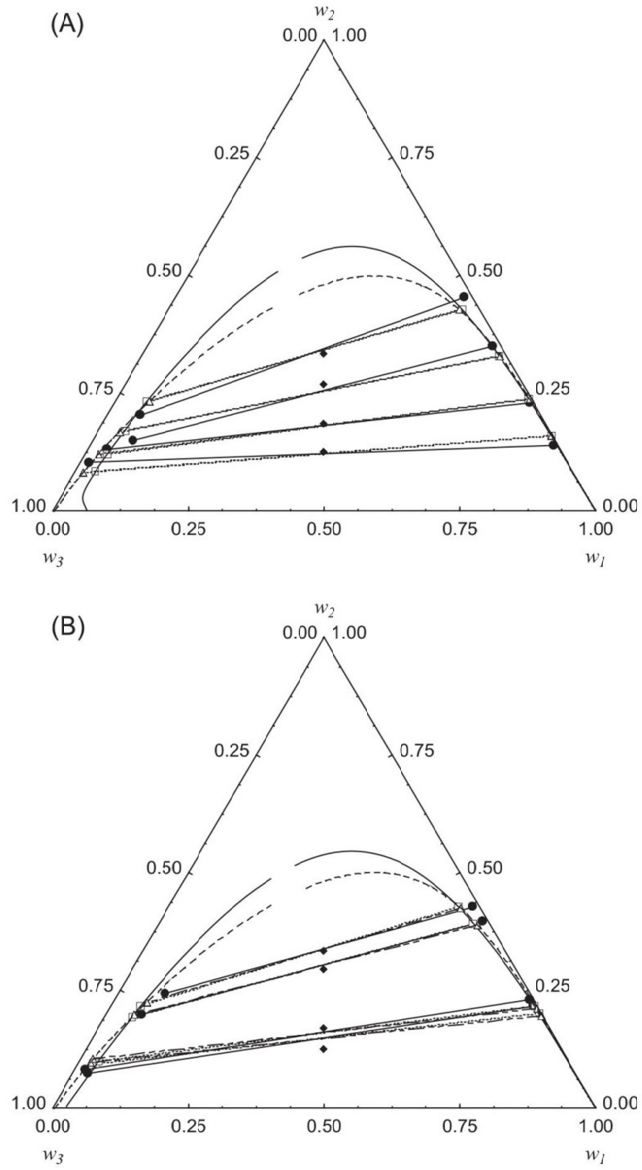


Fig. 4. Ternary diagram for the system water(1) + ethanol(2) + FA-SSAO(3) at T = 303.2 K (A) and T = 323.2 K (B). Experimental (—●—, tie lines; ♦ mixture points), NRTL model (···□···, tie lines; —— binodal) and UNIQUAC model (··△··, tie lines; ·····, binodal).

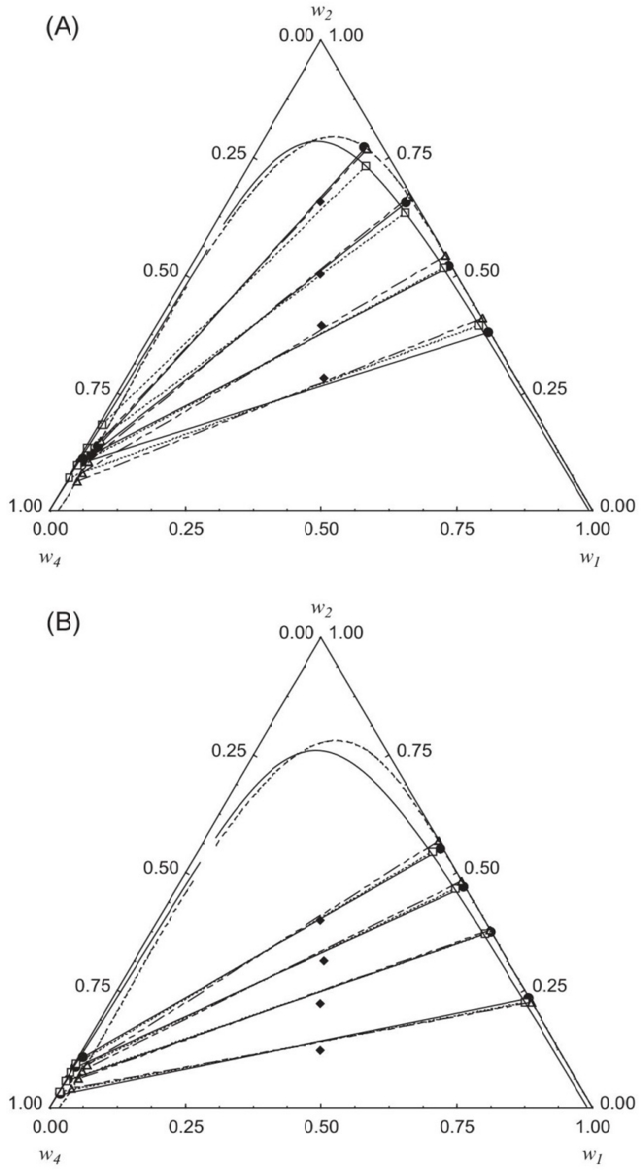


Fig. 5. Ternary diagram for the system water(1) + ethanol(2) + EE-SSAO(3) at T = 303.2 K (A) and T = 323.2 K (B). Experimental (—●—, tie lines; ♦ mixture points), NRTL model (···□···, tie lines; —— binodal) and UNIQUAC model (··△··, tie lines; ·····, binodal).

Table 7
Binary interaction parameters adjusted for the systems water(1) + ethanol(2) + FA-SSAO(3) + EE-SSAO(4) at 303.2 K and 323.2 K.

NRTL	*i–j*	Δg_{ij} (K)	Δg_{ji} (K)	α_{ij}[c]
	1–2[b]	94.20	−10.30	0.2
	1–3	3261.50	−139.09	0.2
	1–4	1838.65	1514.28	0.2
	2–3	1026.59	−1083.57	0.2
	2–4	1831.73	−809.84	0.2
	3–4	NC	NC	
		rmsd (%)	1.79	
UNIQUAC[a]	*i–j*	Δu_{ij} (K)	Δu_{ji} (K)	
	1–2[b]	113.04	22.48	
	1–3	−12.24	857.43	
	1–4	377.41	269.42	
	2–3	123.34	−55.87	
	2–4	−8.25	148.42	
	3–4	NC	NC	
		rmsd (%)	1.67	

[a] *R* and *Q* values obtained from Magnussen et al. [22]. Fitted parameters were $\Delta g_{ij} = (\lambda_{ij} - \lambda_{ii})/R$ and $\Delta u_{ij} = (\lambda_{ij} - \lambda_{jj})/R$.
[b] The pair 1–2 was obtained from Kanda et al. [15].
[c] α_{ij} Fixed for all binary interactions.

indicate the correct tendency of the experimental LLE data obtained in this study.

Figs. 4 and 5 present the ternary diagrams for both systems investigated in this study, showing both the experimental tie lines and the tie lines calculated using both thermodynamic models, NRTL and UNIQUAC. These figures also show the binodal curves predicted using the thermodynamic models. Table 7 shows the binary interaction parameters fitted for both models as well as the *rmsd* (%) values obtained from the correlation to the experimental tie lines. The subgroups and the *r* and *q* values that were used to represent the molecules in the UNIQUAC model are presented in Table 8.

Both activity coefficient models, NRTL and UNIQUAC, were capable of correlating the experimental LLE data for the two ternary systems, giving low *rmsd* (%) values. For the system water(1) + ethanol(2) + FA-SSAO(3), the *rmsd* (%) values were 1.87% for NRTL and 1.84% for UNIQUAC. In the case of the system water(1) + ethanol(2) + EE-SSAO(4), the values were 1.71% for NTRL and 1.49% for UNIQUAC.

The selectivity values of both water and FA-SSAO (S_w and S_{SS}) show that higher ethanol contents were required to increase the mutual solubility between FA-SSAO and water (Table 4). This agrees with previous results, obtained from 298.15 to 323.2 K, for systems containing soybean oil free fatty acids (FFA), ethanol and water. In these systems, the capacity of ethanol to solubilize FFA in the water-rich phase increased as the ethanol content of this phase increased [23–25].

The slopes of the tie lines presented in Fig. 4A and B indicate that ethanol was more soluble in water than in the FA-SSAO-rich phase. This is in agreement with the observation of Bucalá et al. [26] that, in media used for biodiesel production by enzymatic esterification, as the overall water content of the system increases, the ethanol content of the fatty-acid-rich phase decreases.

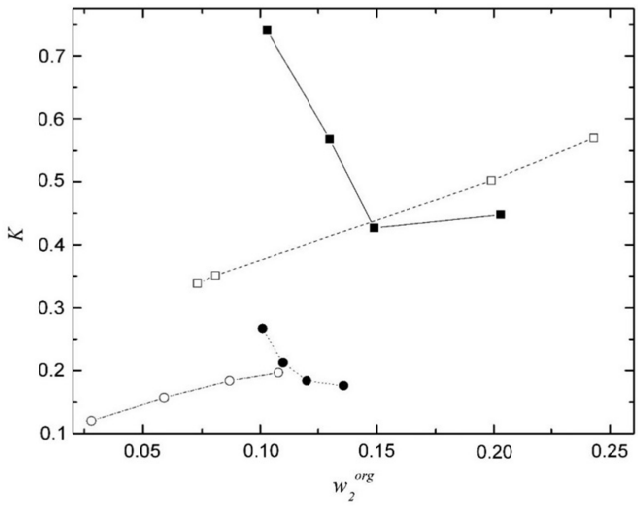


Fig. 6. Ethanol distribution coefficient in function of ethanol mass fraction in organic-rich phases. Water(1) + ethanol(2) + FA-SSAO(3) at $T = 303.2$ K (■) and $T = 323.2$ K (□). Water(1) + ethanol(2) + EE-SSAO(4) at $T = 303.2$ K (●) and $T = 323.2$ K (○).

Our results for LLE, binodal curves and tie lines (Table 6 and Fig. 5A and B) are comparable with those of Mazutti et al. [6], who obtained LLE data for a system comprised of soybean oil ethyl esters + ethanol + water. Their LLE data showed an increase in the solubility of water in soybean ethyl esters as the temperature increased from 303.2 to 333.15 K, and our data show a similar increase in the solubility of water in EE-SSAO as the temperature increases from 303.2 to 323.2 K.

At 303.2 K, as the ethanol content in the organic phase increases, its distribution coefficient decreases (Fig. 6). On the contrary, at 323.2 K, as the ethanol content in the organic phase increases, its distribution coefficient also increases. These observations are true for both systems (i.e., both (1) + (2) + (3) and (1) + (2) + (4)). Both of these thermodynamic behaviors, namely of the ethanol distribution coefficients and of the slopes of the tie lines, indicate that ethanol was more soluble in the water-phase than in the organic phase (i.e., the ester or fatty acid phase), and that this solubility was enhanced at the higher temperature. Moreover, for all overall compositions, the S'_w values at 303.2 K remained above 100, while those at 323.2 K remained above 900, showing a poor solubility of EE-SSAO in the water-rich phase. The S_{EE} values range from 1.563 to 20.408 at 303.2 K and from 9.43 to 13.631 at 323.2 K. The higher values indicate a preference of EE-SSAO to solubilize ethanol rather than water, showing that the selectivity of the EE-SSAO-rich phase in solubilizing ethanol decreased as its the water content increased.

The preference of ethanol for the water-rich phase in comparison to the biodiesel-rich phase, as shown by the *K* values in Tables 5 and 6 and by Fig. 6, is expected, since ethanol can form more hydrogen bonds with water than it can with the biodiesel ethyl esters. It corroborates with the well-known and expected process information that the remaining ethanol is extracted from the

Table 8
Subgroup number of components and *R* and *Q* values used in this work.

Component/subgroup	CH_2COO	COOH	CH_2	CH_3	OH	CH=CH	H_2O
Ethanol	0	0	1	1	1	0	0
Water	0	0	0	0	0	0	1
FA-SSAO	0	1	13.18	1	0	1.24	0
EE-SSAO	1	0	13.18	2	0	1.24	0
R values	1.6764	1.3013	0.6744	0.9011	1.0000	1.1167	0.9200
Q values	1.4200	1.2240	0.5400	0.8480	1.2000	0.8670	1.400

biodiesel phase during the water washing step. This information will be useful in the design of the purification stage, where the hot-water washing step can provide a low ethanol concentration in the biodiesel phase.

Although many authors have produced LLE data for systems involving fatty acids, short chain alcohols, water and the corresponding esters [6–13,15,23–25,27–32], our study presents a new set of experimental data regarding LLE obtained for mixtures containing fatty acids from soybean soapstock acid oil (FA-SSAO) and their corresponding esters (EE-SSAO). The experimental and theoretical thermodynamic data that we have obtained regarding mutual solubility and phase behavior of the reactants and products of the esterification reaction can be used in the modeling and optimization of a low-temperature enzyme-catalyzed esterification process. Additionally, our data can be used in the modeling, analysis, design and optimization of separation processes to produce a high purity biodiesel from this very promising feedstock.

4. Conclusions

We obtained experimental LLE data (binodal curves and tie lines) for the systems water + ethanol + FA-SSAO and water + ethanol + EE-SSAO, at 303.2 and 323.2 K. For the system with FA-SSAO, temperature had little effect on the mutual solubility of the components. In the case of the system with EE-SSAO, the increase of 20 K increased the mutual solubility of the components, as shown by the decrease of area of the biphasic region on the phase diagram. The ethanol distribution coefficients and the values obtained for the selectivity of water, FA-SSAO, and EE-SSAO showed that the water content in the organic phases increased as ethanol content in these phases increased. The two activity coefficient models, UNIQUAC and NTRL, were able to correlate well the experimental ELL data for these highly non-ideal mixtures. The investigations performed in this work may be valuable for the development of a process for the production of biodiesel from fatty acids from soybean soapstock acid oil.

Acknowledgments

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3. CONSIDERAÇÕES FINAIS

Neste trabalho, foi desenvolvido um modelo cinético combinado com efeito de sorção para descrever a esterificação de ácidos graxos com etanol em sistema livre de solvente, catalisada por sólido fermentado com atividade lipolítica. O modelo incorporou isotermas de Langmuir multicomponente para descrever o efeito de sorção dos componentes no sólido fermentado e usou uma equação simplificada da cinética de lipases para descrever a velocidade da reação dependente da composição da fase sorvida. O modelo ajustou-se bem aos dados experimentais obtidos de Soares *et al*. (2015), tanto para conversão global quanto para composições das fases orgânica e sorvida no sólido fermentado.

Também, foram obtidos dados de equilíbrio líquido-líquido (ELL) para os sistemas “ácidos graxos + etanol + água” e “ésteres etílicos + etanol + água” e foi realizada a modelagem termodinâmica destes sistemas com dois modelos de coeficientes de atividade, NRTL e UNIQUAC. Os dados de ELL mostraram que a temperatura teve pouco efeito na solubilidade dos componentes para o primeiro sistema e que um aumento de 20 K na temperatura aumentou a solubilidade dos componentes do segundo sistema estudado. Os valores de coeficientes de distribuição de etanol e os valores de coeficientes de seletividade da água, ácidos graxos e ésteres etílicos mostraram que o teor de água na fase orgânica dos dois sistemas aumentou com o aumento do teor de etanol nestas fases. No que diz respeito à modelagem termodinâmica, os dois modelos de coeficientes de atividade, NRTL e UNIQUAC, correlacionaram bem os dados de ELL experimentais para estes dois sistemas complexos, como foi comprovado pelos baixos valores de *rmsd* obtidos. Além disso, a qualidade dos dados de ELL experimentais foi verificada através das correlações de Hand e Othmer-Tobias, cujos valores de coeficientes de correlação foram superiores a 0,97.

O modelo de cinética combinada com sorção que foi desenvolvido neste trabalho pode ser futuramente melhorado a partir da incorporação de dois fenômenos:

- a desnaturação de lipases por etanol. Como mostrado por Soares *et al*. (2015), para a razão molar de etanol para ácidos graxos de 3:1, a

velocidade de reação, bem como o valor de conversão, foram menores do que as outras razões molares avaliadas, o que pode ser consequência direta da desnaturação da lipase contida no sólido fermentado pela alta concentração de etanol neste sólido.

- a incorporação de um modelo termodinâmico de ELL para descrever a separação das fases no reservatório do sistema usada por Soares *et al*. (2015). Nos experimentos simulados nesta tese, eles agitaram o reservatório do meio reacional que foi recirculado continuamente pelo reator de leito fixo contendo o sólido fermentado. Entretanto, em outros experimentos, eles obtiveram maiores valores de conversão sem agitação do meio reacional, o que permitiu separação das fases orgânica e aquosa. Logo, nestes outros experimentos, o meio reacional que entrava no reator de leito fixo era constituído basicamente de fase orgânica. Este modo de operação aumentou a conversão obtida no sistema e poderia ser descrito, posteriormente, pela integração do modelo de cinética-sorção desenvolvido nesta tese (aplicado ao biorreator de leito fixo), com um modelo de equilíbrio de fases baseado nos dados de ELL obtidos nesta tese (aplicado ao reservatório).

Uma vez aprimorado com estas modificações, o modelo poderá ser usado para guiar a ampliação de escala e a otimização da operação de sistemas de esterificação catalisados por sólidos fermentados com atividade lipolítica.

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